ordinary temperature, and the purer the product, the more rapid the deterioration. This has been found to be true in working with the dilutions of glycerol extracts of the pancreas with water. But in the incubation of trypsin solutions at 40° there does not appear to be much change between 30 minutes and 180 minutes. At a temperature of 50° the weakening is rapid, while at 60° destruction follows.

In agreement with the results of other workers, trypsin is active in the presence of rather large weights of sodium carbonate. A 1% concentration of this salt does not weaken the activity in fibrin digestion at 40°. This corresponds roughly to a hydroxyl strength of 0.01 normal at this temperature. At higher temperatures the action of the carbonate is much more marked, and here we find the effect of the water incubation hastened. In the digestion of fibrin by trypsin 0.2% and 1% sodium carbonate have about the same action.

In these experiments we have been assisted by Mr. George W. Muhleman, to whom we extend our thanks.

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STUDIES IN BACTERIAL METABOLISM XIII-XXX.

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Received June 26, 1913.

XIII. Certain Factors which Influence Bacterial Metabolism.

The chemistry of cellular metabolism is one of decided importance, but one in which the exact details are still largely unknown. The most important results bearing upon this subject up to the present time have been obtained from experiments upon man and the higher animals: in them, metabolic balances have been carried out with a great degree of precision, but the very complexity of the problem, including as it does modifications attributable to an extensive physiological division of labor between cells, tissues and organs, does not permit of more than a very generalized conception of the actual internal mechanism involved. Such experiments have measured the alpha and the omega of the process satisfactorily and furnished much collateral information as the experimental conditions are varied along definit lines: the true inter- and intra-cellular chemistry has not been elucidated up to the present time.

The bacteria offer rather unusual opportunities for the study of unicellular metabolism, for with them it is possible to subject cells of the same kind to careful measurements. The results obtained cannot as yet be applied *in toto* to the cells of the human body, but at least the general principles of cellular metabolism, which these unicellular organisms exhibit, have more than academic interest: they have a practical bearing

upon problems of similar nature in the more complex cellular organizations, for the general principles involved must be phylogenetically related.

Bacterial cells differ in a number of important directions from the cells of more complex individuals: bacterial cells have no morphologic nucleus (although they contain nuclear material): they divide directly by transverse fusion instead of indirectly by mitosis, and very much more rapidly. Bacteria arrive at maturity and reproduce with astonishing rapidity: in some types successive generations may appear at intervals of but fifteen minutes. The absence of sex in the bacteria also is a feature which must be borne in mind. All of these differences must be considered carefully before attempting to generalize from observations on one limited horizon. On the other hand, certain of these factors are important. This very rapid growth of bacteria is an advantage, because the corresponding accumulation of products of their activities bring about changes in their environment of sufficient magnitude to be measured with a fair degree of accuracy before collateral and recessive activities become prominent enough to obscure the results.

There is evidence which indicates that bacterial metabolism and human cellular metabolism have certain fundamental features in common. Physiologists have long known that "carbohydrates spare body nitrogen" in man. A series of quantitative experiments indicates that utilizable carbohydrates protect protein to a very considerable degree in bacteria.[1] In man this sparing action is measured by restricting protein in the diet, substituting carbohydrates for most of it, and showing by nitrogen balances, weight curves and carbon dioxide determinations that equilibrium can be maintained. It is practically impossible in man to accomplish more than the determination of the minimal amounts of carbohydrate and protein which shall be compatible with the maintenance of body weight and to so adjust the respective amounts as to determin the minimal nitrogen requirement. The actual intermediary mechanism of the different cells involved in the process is practically unknown: the results are determined by the food intake, the excretory waste and the physical condition of the patient.

With the bacteria a similar result is accomplished in a somewhat different manner. It is impossible to add just enough nitrogen to keep bacteria in nitrogen equilibrium because the "birth rate" in a culture of bacteria cannot be predicted: hundreds of successive generations occur in the same experiment. On the other hand, it is possible to present bacteria with a *choice* between utilizable carbohydrates and utilizable proteins and observe the result. If the sparing action of carbohydrate for protein is a definit feature of their metabolism, the end products of such an experi-

[1] Kendall and Farmer, *J. Biol. Chem.*, **12**, 1912.

mental digestion should contain evidence of a chemical nature which can be measured both qualitatively and quantitatively. It might be predicted *a priori* that the nature of the products formed by bacterial activity (or at least certain of them) should be similar to certain of those formed during multicellular metabolism—as in man or the higher animals—since in the last analysis all cells must have at least certain characteristics in common. The primary object of the communications which will follow, is to show quantitatively, by analytical data, the effect of utilizable carbohydrate upon the metabolism of protein and protein derivatives by bacteria. A previous series of studies upon certain of the more commonly met with bacteria indicated that those bacteria which could utilize both carbohydrate and protein for katabolic purposes acted upon the former in preference to the latter.[1] Qualitatively the evidence in support of this view is very striking: it has been discussed in some detail in an earlier series of communications, and will not be referred to here.[2] The importance of this sparing action in the limited number of organisms where the qualitative results are particularly noteworthy was so apparent, it has been deemed profitable to continue this line of research to include as many types of bacteria as possible, and to measure comparatively the metabolism of several strains of the same variety of organism to determine the "physiological limits," if such exist, of each "species."

In general, cellular metabolism consists essentially of two distinct phases—the anabolic, constructive or structural phase, and the katabolic, destructive or "fuel" phase. The actual structural material entering into a single bacterial cell is extremely small, being for an average organism which measures about one micron in diameter (0.001 mm.) and 2 microns in length, about 0.000,000,0016 mg. This is determined as follows: a bacterial cell of the dimension given above is a cylinder. Its volume is, therefore (assuming the ends are flat, like a true cylinder), $(0.001)^2 \times 0.7854 \times 0.002 = 0.000,000,00157$ cubic millimeters. The specific gravity of the average bacterial cell has been determined to be approximately 1.038, hence the weight of the bacterial cell would be 0.000,000,0016 mg. Fully 85% of this weight is water, hence the *actual* amount of protein contained in it is very little. There is little doubt, however, but that much more protein is required to build up the bodies of bacteria than appears in the completed cell: the waste in other words exceeds the amount actually utilized. The kind and amount of waste varies with different kinds of bacteria within certain limits. Most bacteria can utilize a variety of nitrogenous substances for structural purposes, the actual chemical composition of the organisms varying somewhat in

[1] *J. Biol. Chem.*, 12, 1912.

[2] Kendall, *J. Med. Research*, 24, 411–24 (1911); 25, 117–87 (1912); *Boston Med. and Surg. J.*, 64, 288–94 (1911); June 5, 1913; *Wisconsin Med. J.*, June, 1913.

response to this variation in food. In any event, the combined structural needs and structural waste are much less in amount than the "fuel needs and fuel waste."

Structural needs are practically fulfilled, aside from losses incidental to the elaboration of enzymes, etc., when the individual cell is morphologically complete. Fuel requirements on the contrary only cease with the death of the organism, or a restriction of its vegetative activities, as, for example, prolonged exposure to cold where the cell hibernates, as it were. The fuel requirement, therefore, is one of comparatively long duration, and for this reason alone it would tend to exceed the structural requirement. For fuel purposes, furthermore, the material acted upon is degraded relatively rapidly, more so by bacteria which are prominent in the economy of nature, where a rapid disintegration of nitrogenous substances is a conspicuous result of their growth, less so by bacteria pathogenic for man and animals, where the process is referable to minute amounts of highly complex poisons derived from the action of the organisms upon the host. In other words, saphrophytic bacteria are more active chemically than pathogenic organisms, generally speaking. The relative proteolytic activities of bacteria can be measured quantitatively with a fair degree of accuracy, taking ammonia formation as an index of proteolysis, for ammonia represents the final step of the degradation of protein nitrogen by the ordinary bacteria, there being no available energy in it. The dete mination of ammonia, however, gives no definit idea of the nature of the intermediary metabolism. Bacteria, which can derive their fuel (katabolic) requirements from either protein (or protein derivatives) or carbohydrate, utilize the carbohydrate in preference to the protein: this is shown by the greater ammonia production in sugar-free media than in corresponding media containing sugar. This would be expected, for it is a well attested physiological fact that the energy of carbohydrates is more easily utilized than that of protein. At the same time, at least a minimal amount of nitrogen must be available for anabolic purposes, because bacteria in common with all living things contain nitrogen in their structure.

In measuring bacterial metabolism, several sources of variation must be considered. These may logically be placed under three heads: (1) those inherent in the bacteria; (2) those inherent in the media in which the bacteria are grown; and (3) those relating to the accuracy of the analytical methods.

1. *Bacterial Variation.*—Bacterial variation may be of three types:

a. Morphological variation, in which some morphological structure may differ from the normal, *e. g.*, the suppression of sporulation in the anthrax bacillus. Morphological variation is for the most part unimportant in this connection.

b. Environmental Activity.—It is frankly conceded that many bacteria grown in artificial media are placed in unnatural conditions: bacteria progressively pathogenic for man or animals for example do not find the same environment in cultures that they are accustomed to in their host. It is obviously impossible for instance to measure the metabolism of typhoid bacilli in the human body, hence bacteria in general must be measured on a common basis, even though it be a somewhat unnatural one for some of them.

c. Vegetative Activity.—The same strain of bacterium does not necessarily grow with the same degree of uniformity at different times, even though the other factors remain the same. Certain cultures of *B. proteus*, for example, have become definitely parasitized on agar through repeated transfers on this medium for several years, and will not develop noticeably in broth. This is an extreme case: most organisms may require preliminary reactivation before their maximum growth can be obtained. Many pathogenic bacteria freshly isolated from their host grow feebly until they become somewhat accustomed to their new environment.

d. Chemical Activity.—The ability to produce characteristic chemical changes may also be temporarily or even permanently lost by bacteria. *B. cloacae* very commonly loses its property of liquefying gelatin. *B. proteus* and *B. coli* may cease to ferment certain of the more complex sugars, although they apparently grow with their original luxuriance.

These deviations from the normal at first sight might seem to render futile any attempts to make accurate comparative observations. As a matter of fact, experience has shown that the variations observed between different bacteria and various strains of the same bacterium are far less than might be expected: indeed, these variations from the normal are relatively insignificant, except for rare instances: here the gross differences would almost certainly attract attention to them.

2. *Variation in Media.*—It is self-evident that comparative studies of bacterial metabolism must be carried out in media of the same qualitative and quantitative composition. Media made from standard ingredients according to a definite formula are fairly uniform in these respects. The media employed in the studies recorded below were made in accordance with such a procedure. This is the best that can be done at the present time. Observations on the effect of definit variations in standard media will be discussed elsewhere. The media used in these experiments were made from meat juice freed from muscle sugar by fermentation with *B. coli* in the usual manner (Smith method) containing in addition one per cent of Witte's peptone. The reaction of this meat juice-peptone broth was adjusted to practical neutrality, then divided into two equal parts. 1% of glucose was added to one-half, then both portions were respectively flasked, 100 cc. to a flask. The flasked broths were then

sterilized simultaneously. The composition of this sugar-free and glucose broth, therefore, was precisely the same except for the added sugar to one-half of it. A series of four flasks each of such sugar-free and sugar-containing broth was inoculated with a reactivated culture of the desired organism, and incubated at 37°. A flask of sugar-free and sugar broth of each organism was examined at the end of 24 hours, three, six and nine days, respectively, according to the procedure outlined below. The necessary controls were examined according to the same scheme.

3. *Accuracy of Analytical Methods.*—The chemical determinations comprise the following:

1. *Free Ammonia.*—The ammonia contained in 2 cc. of broth was determined by the new Folin air current method.[1]

2. *Total nitrogen* was determined according to the Folin-Farmer method.[2] Previous experiments have shown that cultures of ordinary bacteria neither gain nor lose nitrogen, hence this determination was made but once, and then on uninoculated controls.

3. *Formal Titration.*—5 cc. of culture diluted to 50 cc. with distilled water were neutralized to phenolphthalein: 5 cc. of neutral formalin were added, allowed to stand 30 minutes, and again titrated, using 0.1 *N* sodium hydroxide. The results furnished no information of importance, perhaps because of the relatively small amount of amino acids present in the media.

4. *Determination of Reaction.*—5 cc. of culture, diluted with 45 cc. of distilled water, were titrated to neutrality with 0.1 *N* sodium hydroxide or hydrochloric acid, using respectively alizarin, neutral red and phenolphthalein as indicators. The solution was heated to boiling prior to the use of the latter to drive off carbon dioxide. Generally speaking, the results ran parallel with the three indicators, alizarin showing the greatest alkalinity, phenolphthalein the greatest acidity. Neutral red appears to show most closely the point of actual neutrality.

Accuracy of Determinations.—The ammonia and total nitrogen determinations were done in duplicate: the greatest variation allowed in duplicate determinations was 0.1 cc. of 0.5 *N* acid or alkali. This corresponds to a maximum variation of one per cent., or to 1.4 milligrams per 100 cc. broth: that is to say, 1.4 parts in 100,000 parts of broth. The reactions are accurate to $^1/_4$ cc. normal acid or alkali per 100 cc.

The object of the experiments which follow is to show comparatively, by analytical methods, the extent, and to a lesser degree the nature of the sparing action which utilizable carbohydrate exerts for protein or protein derivatives for certain bacteria. Several strains of bacteria representing a majority of the important types are included to show not only

[1] Folin and Macallum, *J, Biol. Chem.*, **11**, 523–5 (1912).

[2] Folin and Farmer, *Ibid.*, —, 493–501.

the prominent chemical characteristics of these types, but also the extent of the variation which is likely to be met with among organisms of the same "species." The results presented in these tables indicate the nature and extent of these variations, as well as the sparing action which utilizable carbohydrate exerts for protein, shielding the latter from bacterial attack. The results are expressed in terms of 100 cc. of broth.

It will be remembered that both the sugar-free and sugar-containing broths were placed in 250 cc. Erlenmeyer flasks: this provides a maximum exposure of the free surface of the media to the air. Pure protein solutions are not as a rule decomposed by bacteria in the absence of oxygen: this free surface exposure, therefore, gives bacteria, even in sugar-containing media, a maximum opportunity to utilize protein in preference to the sugar. They do not necessarily have to derive their oxygen from the sugar. The observed sparing action of the carbohydrate for protein, therefore, is all the more striking.

The essential cultural characters of the various organisms studied are tabulated under the appropriate groups. The arrangement of bacteria in groups is an important feature for two reasons: it shows the limits of variability which have been met with in these groups and permits of a comparison of certain cultural and chemical peculiarities which are characteristic and of diagnostic importance.

Legend: For brevity, the following abbreviations are used in the tabulation of the cultural characteristics of the bacteria studied.

Glucose, Lactose, Sucrose and Mannite Broths.

+ = acid formation: sugar fermented.
— = alkali formation: sugar not fermented.
g = gas formation: acid is always produced coincidently with gas.

Milk.

+ = acid formed: $+_s$ slight amount of acid formed.
— = alkali formed; sugar unfermented.
± = initial acidity, terminal alkalinity. Slight amount of glucose present in fresh milk is fermented completely, giving rise to initial acidity; then protein is attacked, alkali formed, and initial acid is neutralized.
g = gas formation.
c = coagulation, due either to acid or enzyme.
p = peptonization.

Gelatin.

+ = liquefaction.
— = no liquefaction.

Indol.

+ = indol formation.
— = no indol formed.

Motility.

+ = motile.
— = non-motile.

XIV. Diphtheria Bacillus.

The true diphtheria bacillus produces an extremely potent extracellular toxin, both in the human body and, under perfectly definit conditions, in artificial media as well. The absorption of this toxin in sufficient amounts causes the essential features of the diphtheria syndrome in man.

Culturally diphtheria bacilli are inactive, resembling the Shiga type of the dysentery bacillus in this respect. These organisms form acid in glucose broth, and slight amounts of acid in fresh steril milk: otherwise they ferment none of the ordinary sugars.

	Glucose.	Lactose.	Sucrose.	Mannite.	Milk.	Gelatin.	Motility.	Indol.
B. diphtheriae.....	+	—	—	—	$+_S$	—	—	—

In sugar-free broth diphtheria bacilli produce small amounts of ammonia, distinctly less amounts in the corresponding glucose broth. This general type of relative chemical inactivity is exhibited by a majority of the organisms progressively pathogenic for man. The question arises—is there any significance to be attributed to this slight sparing action which the glucose exhibits for the protein constituents of the broth, the former shielding the latter from bacterial breakdown?

The facts are these: in *sugar-free* broth certain strains of the diphtheria bacillus produce a toxin of such potency that 1/1000 cc. of the nine-day broth culture freed from bacteria by filtration through unglazed porcelain kills a 250 gram pig in about four days. In sugar-free broth it will be remembered, bacteria must derive both their structural (anabolic) and vegetative or fuel (katabolic) needs from the protein constituents of the broth: there is no other available source of energy. The ammonia production observed in this broth represents the combined nitrogen waste of the structural and fuel requirements. This broth is strongly toxic.

In glucose broth, that is, broth of precisely the same initial composition and reaction as the sugar-free broth but containing 1% of glucose in addition, no measurable amounts of toxin are found, as Theobald Smith showed conclusively many years ago.[1] Several cubic centimeters of the filtrate of this glucose-broth culture of diphtheria bacilli are innocuous for guinea pigs. That is to say—several hundred times more of this glucose broth than of the corresponding sugar-free broth, which is sufficient to kill a guinea pig in four days, can be injected into guinea pigs without appreciable effect.

The diphtheria bacilli obtain their structural nitrogenous requirement from protein in glucose broth as they do in sugar-free broth: the fuel requirement, however, is obtained from the glucose in sugar broth. The ammonia increase in glucose broth, therefore, is referable to the nitrogenous waste of structural needs only: that observed in sugar-free broth

[1] Theobald Smith, *Trans, Assoc. Am. Physiologist*, 1896.

Organism.	Days.	Plain broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	Per cent. NH_3/total N_2.	Glucose broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	Per cent. NH_3/total N_2.
B. diphtheriae, 1	1	+0.20	0.00	0.00	23.80	+0.70	0.22	+0.20	+0.60	+0.40	23.10	0.00	0.00
	3	0.00	—0.10	+0.20	24.50	+1.40	0.45	+2.00	+2.30	+2.00	23.10	0.00	0.00
	6	—0.20	—0.50	—0.10	25.20	+2.10	0.67	+1.90	+2.00	+1.90	23.80	+0.70	0.23
	9	—0.60	—0.50	—0.20	26.60	+3.50	1.11	+2.10	+2.10	+2.20	24.50	+1.40	0.46
B. diphtheriae, 2	1	+0.10	0.00	0.00	23.80	+0.70	0.22	+0.20	+0.20	+0.20	23.10	0.00	0.00
	3	0.00	0.00	+0.10	25.20	+2.10	0.67	+1.60	+2.10	+1.90	23.10	0.00	0.00
	6	—0.50	—0.20	—0.20	25.90	+2.80	0.89	+1.90	+2.20	+2.30	23.80	+0.70	0.23
	9	—0.40	—0.80	—0.40	27.30	+4.20	1.34	+2.10	+2.40	+2.50	24.50	+1.40	0.46
B. diphtheriae, 3	1	0.00	0.00	+0.30	18.90	0.00	0.00	+0.20	+0.60	+1.80	18.20	—0.70	0.25
	3	0.00	+0.10	+0.20	20.30	+1.40	0.51	+2.70	+2.40	+3.20	18.90	0.00	0.00
	6	—0.40	—0.40	—0.30	23.80	+4.90	1.80	+2.80	+3.40	+3.30	18.90	0.00	0.00
	9	—0.50	—0.50	—0.20	24.50	+5.60	2.05	+3.30	+3.70	+3.70	19.60	+0.70	0.25
B. diphtheriae, 8	1	+0.20	0.00	+0.10	23.80	+0.70	0.22	+0.10	+0.10	+0.20	23.10	0.00	0.00
	3	—0.10	—0.10	+0.20	25.20	+2.10	0.67	+1.40	+1.80	+1.70	23.10	0.00	0.00
	6	—0.40	—0.40	—0.10	27.30	+4.20	1.34	+1.80	+2.30	+2.60	23.80	+0.70	0.23
	9	—0.30	—0.30	—0.30	26.60	+3.50	1.11	+2.10	+2.50	+2.30	24.50	+1.40	0.46
B. diphtheriae, *a*	1	+0.20	0.00	+0.10	23.80	+0.70	0.22	+0.20	+0.60	+1.50	23.10	0.00	0.00
	3	—0.70	—0.40	—0.40	24.50	+1.40	0.45	+2.10	+2.80	+2.90	23.10	0.00	0.00
	6	—0.30	—0.40	—0.30	25.20	+2.10	0.67	+1.80	+2.50	+2.60	23.10	0.00	0.00
	9	—0.40	—0.60	—0.60	25.20	+2.10	0.67	+3.00	+2.70	+2.60	23.80	+0.70	0.23
B. diphtheriae, *b*	1	+0.20	0.00	+0.20	23.80	+0.70	0.22	+0.10	+0.70	+0.50	23.10	0.00	0.00
	3	—0.30	—0.30	—0.20	25.20	+2.10	0.67	+2.40	+2.60	+2.70	23.10	0.00	0.00
	6	—0.30	—0.60	—0.30	25.20	+2.10	0.67	+2.20	+2.70	+2.70	23.80	+0.70	0.23
	9	—0.30	—0.50	—0.40	26.60	+3.50	1.11	+2.50	+2.60	+2.70	23.80	+0.70	0.23

Organism.	Days.	Plain broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.	Glucose broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.
B. diphtheriae, c.....	1	—0.20	0.00	+0.20	23.80	+0.70	0.22	+0.30	+0.70	+0.40	23.10	0.00	0.00
	3	—0.70	—0.50	—0.20	23.80	+0.70	0.22	+2.20	+2.90	+2.70	23.10	0.00	0.00
	6	—0.20	—0.50	—0.20	24.50	+1.40	0.45	+3.00	+3.20	+3.20	23.80	+0.70	0.23
	9	—0.40	—0.50	—0.40	25.90	+2.80	0.89	+3.10	+3.30	+3.10	24.50	+1.40	0.46
B. diphtheriae, d.....	1	—0.20	0.00	+0.10	23.80	+0.70	0.22	+0.40	+0.70	+0.20	23.10	0.00	0.00
	3	—0.20	—0.30	—0.10	23.80	+0.70	0.22	+2.10	+2.50	+2.50	23.10	0.00	0.00
	6	—0.30	—0.30	—0.10	24.50	+1.40	0.45	+2.00	+2.50	+2.60	23.80	+0.70	0.23
	9	—0.40	—0.60	—0.50	26.60	+3.50	1.11	+2.10	+2.90	+2.80	24.50	+1.40	0.45
B. diphtheriae, 6.....	1	+0.10	0.00	+0.10	23.80	+0.70	0.22	+0.20	+0.70	+0.60	23.10	0.00	0.00
	3	—0.50	—0.30	—0.30	23.80	+0.70	0.22	+2.40	+2.70	+3.00	23.10	0.00	0.00
	6	—0.30	—0.50	—0.20	25.20	+2.10	0.67	+3.10	+2.80	+3.10	23.80	+0.70	0.23
	9	—0.60	—0.60	—0.50	25.90	+2.80	0.89	+3.00	+2.80	+3.10	23.80	+0.70	0.23
B. diphtheriae, 7	1	+0.20	0.00	+0.10	23.10	0.00	0.00	+0.10	+0.10	+0.10	23.10	0.00	0.00
	3	—0.10	—0.20	0.00	23.80	+0.70	0.22	+2.30	+2.80	+2.80	23.80	+0.70	0.23
	6	—0.60	—0.80	—0.10	24.50	+1.40	0.45	+2.30	+2.70	+2.90	23.80	+0.70	0.23
	9	0.00	—0.40	—0.10	25.90	+2.80	0.89	+2.20	+2.20	+2.40	24.50	+1.40	0.45
B. diphtheriae, 9.....	1	+0.20	0.00	+0.10	23.10	0.00	0.00	+1.80	+2.00	+1.40	23.10	0.00	0.00
	3	—0.10	—0.20	—0.10	23.10	0.00	0.00	+2.10	+2.70	+2.40	23.10	0.00	0.00
	6	—0.50	—0.60	0.00	24.50	+1.40	0.45	+1.90	+2.70	+2.80	23.80	+0.70	0.23
	9	—0.60	—0.60	—0.20	25.90	+2.80	0.89	+2.10	+3.20	+3.00	24.50	+1.40	0.45

is the combined structural and fuel waste. The latter is greater than the former.

Inasmuch as approximately the same number of diphtheria bacilli are found in the sugar-free and sugar-containing broths, respectively, the signification of the sparing action becomes manifest. Toxin formation by the diphtheria bacillus is associated with the katabolic utilization of protein (or protein derivatives) for it is not produced coincidently with the anabolic utilization of protein.

The sparing action of utilizable carbohydrate for protein therefore, even if it is apparently small in amount, as is the case with the diphtheria bacillus, is in reality of great importance in determining the nature of the activity of these organisms.

The cultures studied here were obtained from the following sources: Bacillus III was sent to us by Professor Theobald Smith; Number VIII is an organism isolated by Dr. Park of the Research Laboratory of New York City, and is the culture which is widely used throughout the world for making diphtheria toxin. The other organisms were isolated from an epidemic of diphtheria which occurred in Chicago.

XV. Dysentery Group.

Bacillary dysentery is a type of severe intestinal infection occurring in certain parts of the Orient, notably Japan. It also occurs sporadically and endemically in the United States and other parts of the world. The discovery of the causative organism of epidemic dysentery (*B. dysenteriae*) by Shiga, which bears his name, has led to an immense amount of work in many countries upon the etiology of dysentery. The result of this work has been the addition of a considerable number of closely related organisms, of which the Shiga and Flexner types appear to be the most important, to the list of bacteria pathogenic for man. The most noteworthy cultural difference between the Shiga and Flexner types is the inability of the former to ferment mannite. Their reactions in the customary media follow:

	Glucose.	Lactose.	Sucrose.	Mannite.	Milk.	Gelatin.	Motility.	Indol.
B. dysenteriae, Shiga.....	+	—	—	—	±	—	—	—
B. dysenteriae, Flexner...	+	—	—	+	±	—	—	—

The Shiga bacillus is usually the more virulent of the two, producing striking anatomical lesions in the intestinal tract, and generally speaking the Shiga bacillus is the organism more commonly found in epidemic dysentery; the Flexner bacillus is more often met with in sporadic cases of bacillary dysentery. Chemically the two types appear to be very much alike except that the Shiga bacillus produces a certain amount of acid in sugar-free broth. This has been noticed before by one of us.[1] So far as their chemical activity is concerned the dysentery bacillus,

[1] Kendall and Farmer, *J. Biol Chem.*, 12, 16 (1912).

Organism.	Days.	Plain broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.	Glucose broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.
B. dysenteriae, Shiga.....	1	—0.10	+0.20	+0.20	18.20	0.00	0.00	+2.20	+2.70	+2.00	17.50	—0.70	—0.24
	3	—0.10	+0.20	+0.40	20.30	+2.10	0.71	+2.50	+2.80	+2.20	18.20	0.00	0.00
	6	—0.10	—0.30	+0.10	21.00	+2.80	0.95	+2.40	+2.90	+2.50	18.20	0.00	0.00
	9	—0.40	—0.30	0.00	22.40	+4.20	1.43	+2.50	+2.80	+2.40	18.20	0.00	0.00
B. dysenteriae, Flexner....	1	—0.10	+0.10	+0.10	18.20	0.00	0.00	+2.10	+2.70	+1.90	17.50	—0.70	0.24
	3	—0.60	+0.10	0.00	19.60	+1.40	0.48	+2.00	+2.70	+2.00	18.20	0.00	0.00
	6	—0.70	—0.20	—0.30	21.00	+2.80	0.95	+2.10	+2.60	+2.00	18.20	0.00	0.00
	9	—0.80	—0.20	—0.30	21.00	+2.80	0.95	+2.20	+2.30	+2.00	18.20	0.00	0.00
B. dysenteriae, Flexner....	1	—0.30	+0.10	0.00	17.50	+0.70	0.26	+1.80	+0.60	—0.10	16.80	0.00	0.00
	3	—0.20	+0.10	0.00	18.20	+1.40	0.51	+1.90	+2.60	+2.10	16.80	0.00	0.00
	6	—0.30	+0.10	0.00	18.90	+2.10	0.77	+1.90	+2.60	+2.30	16.80	0.00	0.00
	9	—0.50	+0.20	+0.20	20.30	+3.50	1.32	+1.90	+2.50	+2.30	16.80	0.00	0.00
B. dysenteriae, Flexner I...	1	0.00	+0.20	0.00	18.20	0.00	0.00	+2.20	+2.70	+1.90	17.50	—0.70	0.24
	3	—0.60	+0.10	0.00	18.20	0.00	0.00	+2.40	+2.80	+2.00	18.20	0.00	0.00
	6	—1.00	0.00	—0.30	21.70	+3.50	1.19	+2.30	+2.30	+2.20	18.20	0.00	0.00
	9	—1.10	—0.10	—0.40	22.40	+4.20	1.43	+2.60	+2.70	+2.70	18.20	0.00	0.00
B. dysenteriae, Flexner II..	1	0.00	+0.20	+0.10	18.20	0.00	0.00	+2.10	+2.10	+2.10	17.50	—0.70	0.24
	3	—0.40	+0.10	—0.10	19.60	+1.40	0.48	+2.20	+2.60	+2.20	18.20	0.00	0.00
	6	—0.90	—0.30	—0.30	20.30	+2.10	0.71	+2.30	+2.70	+2.30	18.20	0.00	0.00
	9	—1.00	—0.20	—0.20	21.00	+2.80	0.95	+2.70	+2.70	+2.60	18.20	0.00	0.00
B. dysenteriae, Flexner III.	1	—0.10	+0.10	0.00	18.20	0.00	0.00	+2.10	+2.00	+2.10	17.50	—0.70	0.24
	3	—0.50	+0.10	—0.10	19.60	+1.40	0.48	+2.10	+2.50	+2.10	18.20	0.00	0.00
	6	—0.70	—0.20	—0.30	20.30	+2.10	0.71	+2.30	+2.80	+2.30	18.20	0.00	0.00
	9	—0.80	—0.10	—0.20	20.30	+2.10	0.71	+2.60	+3.00	+2.80	18.20	0.00	0.00

typhoid bacillus and *Bacillus alcaligenes* are strikingly alike, all of them forming minimal amounts of free ammonia. These organisms are also very much alike culturally. The sparing action of carbohydrate for protein is clearly brought out in the chemical analyses. There is evidence which indicates that the Shiga bacillus does not form appreciable amounts of toxin in glucose broth, thus showing again the importance of the sparing action of utilizable carbohydrate for protein.

XVI. Typhoid Group.

The typhoid bacillus is one of the best known of the bacteria specifically pathogenic for man. Culturally it is closely related to the dysentery bacilli, and *B. alcaligenes*. One of its striking cultural characteristics is the formation of a permanently acid reaction in milk. Certain strains have been reported from time to time which are said to produce a progressive alkalinity in this medium: however, the strains studied in this series agree chemically with the members of the dysentery group in forming

	Glucose.	Lactose.	Sucrose.	Mannite.	Milk.	Gelatin.	Indol.	Motility.
B. typhosus.....	+	—	—	+	+	—	—	+

but little ammonia in sugar-free broth, smaller amounts in glucose broth, thus showing the sparing action of the carbohydrate for protein. This sparing action of the sugar, may, theoretically at least, be taken advantage of in the dietary treatment of typhoid fever. A continuous excess of sugar in the intestinal tract should prevent to a very considerable extent the action of the typhoid bacillus upon the intestinal tissues, and a discontinuation in the toxic symptoms, and inasmuch as it is possible to maintain a definit concentration of glucose in the blood, the same sparing action should be exhibited in the body tissues as well. Clinical-chemical observations on a series of cases of typhoid fever indicate that this possibility is apparently realized.[1]

XVII. Bacillus Alcaligenes Group.

Bacillus alcaligenes resembles the members of the typhoid-dysentery group culturally except that it is said to ferment no sugars. This statement is grossly correct, but there is evidence which seems to indicate that the organism in the presence of oxygen can derive a certain minimal amount of energy from glucose (or possibly from some unknown impurity of glucose which is present in it in very small amount) as is shown by the increased luxuriance of growth in glucose media, and consequently by the slight but definit increase of ammonia production in glucose broth above that seen in sugar-free broth. No measurable amount of acid is produced in glucose and no gas. This observation has been confirmed not only by the organisms studied here, but also by those in previous experiments of like nature.[2] Whether this slight action of *Bacillus alcaligenes* upon

[1] Coleman and Shaffer, *Arch. Int. Medicine*, 4, 538–600 (1909).

[2] Kendall and Farmer, *J. Biol. Chem.*, **12**, 467 (1912).

Organism.	Days.	Plain broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.	Glucose broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.
B. typhosus, I	1	+0.40	0.00	—0.10	24.50	+ 1.40	0.46	+3.00	+3.10	+3.00	23.10	+0.70	0.26
	3	+0.10	—0.10	—0.10	25.20	+ 2.10	0.70	+3.20	+3.20	+3.00	22.40	0.00	0.00
	6	—0.70	—0.20	—0.30	25.90	+ 2.80	0.95	+3.30	+3.40	+3.00	21.70	—0.70	0.26
	9	—1.00	—0.30	—0.20	27.30	+ 4.20	1.40	+3.40	+3.50	+3.00	21.70	—0.70	0.26
B. typhosus, IA	1	+0.30	0.00	0.00	24.50	+ 1.40	0.46	+2.90	+3.20	+2.80	23.10	+0.70	0.26
	3	0.00	—0.20	0.00	25.20	+ 2.10	0.70	+3.20	+3.30	+2.80	22.40	0.00	0.00
	6	—0.30	—0.10	—0.30	25.20	+ 2.10	0.70	+3.20	+3.30	+2.80	21.70	—0.70	0.26
	9	—0.90	—0.30	—0.40	28.00	+ 4.90	1.63	+3.30	+3.40	+2.90	21.70	—0.70	0.26
B. typhosus, A	1	0.00	0.00	+0.10	19.60	+ 0.70	0.25	+1.40	+2.60	+2.60	18.20	0.00	0.00
	3	—0.60	—0.30	—0.10	23.80	+ 4.90	1.80	+2.90	+3.10	+2.80	18.20	0.00	0.00
	6	—1.20	—0.70	—0.30	31.50	+12.60	4.62	+2.90	+3.20	+2.80	18.90	+0.70	0.25
	9	—1.40	—0.80	—0.70	29.40	+10.50	3.79	+3.00	+2.90	+3.00	19.60	+1.40	0.50
B. typhosus, Mann	1	+0.10	0.00	+0.10	19.60	+ 0.70	0.25	+1.40	+2.60	+2.50	18.20	0.00	0.00
	3	—0.90	—0.20	—0.20	21.00	+ 2.10	0.75	+2.80	+2.90	+2.50	18.90	+0.70	0.25
	6	—1.40	—0.70	—0.30	23.80	+ 4.90	1.80	+2.90	+3.00	+2.70	18.90	+0.70	0.25
	9	—1.20	—0.90	—0.30	24.50	+ 5.60	2.05	+2.90	+3.10	+2.70	19.60	+1.40	0.50

B. typhosus, I B.....	1	0.00	—0.10	+0.10	19.60	+0.70	0.25	+2.10	+2.80	+2.60	18.20	0.00	0.00
	3	—1.00	—0.20	—0.20	20.30	+1.40	0.51	+2.80	+3.10	+2.80	18.90	+0.70	0.25
	6	—1.20	—0.60	—0.40	24.50	+5.60	2.05	+2.80	+3.30	+2.80	18.90	+0.70	0.25
	9	—1.40	—0.80	—0.70	27.30	+8.40	3.58	+2.90	+3.30	+2.90	18.90	+0.70	0.25
B. typhosus, II.....	1	+0.20	0.00	+0.30	19.60	+0.70	0.25	+1.70	+2.50	+2.60	18.20	0.00	0.00
	3	—0.20	+0.20	+0.10	20.30	+1.40	0.51	+2.90	+3.40	+2.90	18.20	0.00	0.00
	6	—0.60	—0.10	—0.10	23.80	+4.90	1.80	+3.10	+3.60	+2.90	18.90	+0.70	0.25
	9	—0.70	—0.40	—0.20	25.90	+6.00	2.20	+3.10	+3.60	+2.90	19.60	+1.40	0.50
B. typhosus, I C.....	1	—0.10	+0.20	0.00	28.00	+3.50	0.94	+2.70	+3.70	+2.90	24.50	—0.70	0.19
	3	—1.10	+0.10	—0.20	29.40	+4.90	1.32	+3.00	+4.00	+3.30	24.50	—0.70	0.19
	6	—1.60	+0.10	—0.10	30.80	+6.30	1.70	+3.20	+3.90	+3.80	26.60	+1.40	0.38
	9	—1.30	+0.10	0.00	30.80	+6.30	1.70	+3.60	+4.90	+3.60	26.60	+1.40	0.38
B. typhosus, I D.....	1	—0.30	0.00	+0.10	28.00	+3.50	0.94	+2.70	+3.90	+3.30	25.20	0.00	0.00
	3	—1.20	+0.20	+0.10	28.70	+4.20	1.13	+2.90	+4.10	+3.50	23.80	—1.40	0.38
	6	—1.70	0.00	—0.10	30.80	+6.30	1.70	+3.10	+4.50	+3.20	26.60	+1.40	0.38
	9	—1.40	+0.10	—0.10	30.80	+6.30	1.70	+3.00	+4.70	+3.50	27.30	+2.10	0.57
B. typhosus, Dorset.....	1	+0.30	0.00	0.00	23.80	+0.70	0.22	+3.30	+2.90	+2.80	23.10	0.00	0.00
	3	—0.30	—0.20	—0.30	24.50	+1.40	0.45	+3.20	+2.80	+3.10	23.10	0.00	0.00
	6	—0.60	—0.30	—0.50	25.90	+2.80	0.89	+3.30	+3.20	+2.90	23.10	0.00	0.00
	9	—1.10	—0.60	—0.60	28.70	+5.60	1.75	+3.40	+3.20	+3.10	23.10	0.00	0.00

Organism.	Days.	Plain broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.	Glucose broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.
B. alcaligenes	1	—0.50	—0.60	—0.60	18.90	0.00	0.00	—0.80	—0.60	—0.70	18.90	0.00	0.00
	3	—1.10	—0.80	—1.00	18.90	0.00	0.00	—1.40	—0.80	—1.10	16.80	—2.10	0.75
	6	—1.80	—1.00	—1.00	19.60	+0.70	0.26	—2.60	—1.20	—1.20	20.30	+1.40	0.50
	9	—1.80	—1.20	—1.10	22.40	+3.50	1.28	—2.30	—1.00	—1.10	24.50	+5.60	2.00
B. alcaligenes	1	—0.50	—0.20	—0.30	15.40	0.00	0.00	—0.70	—0.20	—0.60	16.10	0.00	0.00
	3	—1.20	—0.80	—0.60	16.10	+0.70	+0.25	—1.10	—0.90	—1.00	16.10	0.00	0.00
	6	—1.70	—1.10	—0.80	18.20	+2.80	+1.00	—2.00	—0.90	—1.10	16.80	+0.70	0.22
	9	—1.80	—1.30	—0.90	18.90	+3.50	+1.25	—2.00	—1.30	—1.20	21.00	+4.90	1.52

glucose represents a vestigial fermentative power or an adventitious activity cannot be definitely stated at this time. Culturally the organisms show the following characteristics:

	Glucose.	Lactose.	Sucrose.	Mannite.	Milk.	Gelatin.	Motility.	Indol.
B. Alcaligenes.....	—	—	—	—	—	—	+	—

The relatively slight action of these organisms on protein, and its general negative activity suggests its relation to organisms of the typhoid-dysentery group. Occasional cases of intestinal infection by this organism, causing typhoidal symptoms, support this view. Inasmuch as *B. alcaligenes* does not produce acid in glucose, there is no sparing action of this sugar for protein as is manifested by the formation of as much ammonia in sugar broth as is found in sugar-free broth under the same conditions.

XVIII. Haemorrhagic Septicemia Group.

The organisms studied in this group comprise *B. cuniculicida*, (snuffles of the rabbit) and Swine Plague Number VIII (*B. Suisepticus*) which were obtained from Professor Theobald Smith; Swine Plague, N. Y., from Professor Winslow and *B. avisepticus* from the university of Chicago. *B. pestis*, the bacillus of human and rat plague, was not included. Culturally these organisms grow rather slowly, producing acid but no gas in glucose, sucrose, mannite and milk. Neither acid nor gas was observed in lactose. Morphologically one of the striking characteristics is bipolar staining when colored with Löffler's methylene blue. One of the most noteworthy chemical characteristics of these organisms is their ability to form considerable amounts of indol. This indicates that the organisms are able to utilize the alanin radical of tryptophane, a property possessed by a very few progressively pathogenic organisms. The cultural characteristics of *B. cuniculicida* are typical for the group and they are as follows:

	Glucose.	Lactose.	Sucrose.	Mannite.	Milk.	Gelatin.	Motility.	Indol.
B. cuniculicida....	+	—	+	+	+	—	—	+

The chemical analyses tabulated below show that the organisms produce but little free ammonia in sugar-free broth, decidedly less so in glucose broth, showing the sparing action of the glucose for protein. The small amount of ammonia formed in plain broth again manifests the comparative value of this determination in cultures of progressively pathogenic organisms where the amount formed is almost invariably small.

XIX. The Paratyphoid Group.

This group is an extensive one, including organisms which produce a variety of lesions in man and many of the higher animals. In man the more important diseases are meat poisoning, caused usually by *B. enteritidis*, and paratyphoid fever. Paratyphoid fever is said to be of two types, very closely related clinically, the one associated with the proliferation of *B. paratyphosus-α*, the other with *B. paratyphosus-β*. This distinction

Organism.	Days.	Plain broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3 total/N_2. Per cent.	Glucose broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.
Snuffles A	1	0.00	+1.00	0.00	19.60	0.00	0.00	+0.80	+1.40	0.00	19.60	0.00	0.00
	3	—1.00	+0.20	—0.10	19.60	0.00	0.00	+0.10	+1.30	+0.20	18.90	—0.70	0.20
	6	—1.20	0.00	—0.30	21.70	+2.10	0.63	+0.30	+1.60	+0.30	19.60	0.00	0.00
	9	—1.60	—0.20	—0.50	24.50	+4.90	1.46	+0.30	+2.40	+0.50	19.60	0.00	0.00
Snuffles B	1	—0.80	+0.10	0.00	18.90	0.00	0.00	+0.60	+1.70	+1.40	18.90	0.00	0.00
	3	—0.60	0.00	—0.10	19.60	+0.70	0.26	0.00	+1.40	+1.40	18.20	—0.70	—0.26
	6	—0.30	—0.40	—0.40	20.30	+1.40	0.51	+1.70	+1.60	+1.80	18.90	0.00	0.00
	9	—0.50	—0.40	—0.40	23.10	+4.20	1.54	+1.30	+2.00	+1.70	18.90	0.00	0.00
B. aviseptieus	1	+0.10	0.00	0.00	24.50	+1.40	0.45	+2.40	+2.80	+2.70	23.10	0.00	0.00
	3	—0.20	—0.40	0.00	25.90	+2.80	0.89	+2.00	+2.60	+2.50	23.10	0.00	0.00
	6	—0.40	—0.30	—0.20	28.70	+5.60	1.78	+2.10	+2.90	+2.80	23.10	0.00	0.00
	9	—0.50	—0.30	—0.50	29.40	+6.30	2.00	+2.30	+2.80	+2.50	24.50	+1.40	0.46
Swine plague VIII *a*	1	0.00	+0.90	+0.10	16.90	0.00	0.00	+1.50	+1.70	+1.40	19.60	0.00	0.00
	3	—1.00	+0.30	+0.10	16.90	0.00	0.00	+1.40	+2.50	+1.70	19.60	0.00	0.00
	6	—1.20	—0.10	—0.40	21.70	+2.10	0.63	+1.40	+2.30	+1.60	19.60	0.00	0.00
	9	—2.20	0.00	—0.50	23.80	+4.20	1.25	+1.80	+2.30	+1.90	19.60	0.00	0.00

Swine plague VIII *b*.....	1	0.00	0.00	+0.10	19.60	+0.70	0.26	+1.40	+1.50	+1.10	19.60	+0.70	0.26
	3	—0.20	—0.20	+0.10	19.60	+0.70	0.26	+1.30	+1.60	+1.40	18.90	0.00	0.00
	6	—0.60	—0.20	—0.30	20.30	+1.40	0.51	+1.80	+1.90	+1.60	19.60	+0.70	0.26
	9	—0.60	—0.20	—0.30	24.50	+5.60	2.05	+1.50	+1.60	+1.70	19.60	+0.70	0.26
Swine plague, N. Y., *a*.....	1	—0.30	+0.60	—0.20	19.60	0.00	0.00	+0.80	+1.00	+0.20	20.30	+0.70	0.20
	3	—1.10	+0.40	—0.20	19.60	0.00	0.00	+0.40	+2.20	+1.30	18.90	—0.70	0.20
	6	—0.60	—0.20	—0.30	20.30	+0.70	0.21	+1.00	+2.70	+1.80	19.60	0.00	0.00
	9	—1.80	+0.10	—0.40	21.70	+2.10	0.63	+1.20	+2.30	+1.80	18.90	—0.70	0.20
Swine plague, N. Y., *b*.....	1	0.00	0.00	+0.10	18.90	—0.00	0.00	+1.00	+1.70	+1.20	18.20	—0.70	0.26
	3	—0.60	—0.10	—0.20	19.60	+0.70	0.26	+1.30	+1.80	+1.60	18.90	0.00	0.00
	6	—0.30	—0.40	—0.40	20.30	+1.40	0.51	+1.30	+2.10	+1.50	19.60	+0.70	0.26
	9	—0.50	—0.50	—0.50	23.10	+4.20	1.54	+1.80	+1.80	+1.70	19.60	+0.70	0.26
Swine plague, N. Y., *c*.....	1	—0.10	0.00	+0.10	16.80	+0.70	0.24	+1.10	+1.80	+1.40	16.80	+0.70	0.24
	3	—0.10	+0.40	0.00	16.80	+0.70	0.24	+2.70	+3.10	+1.80	16.80	+0.70	0.24
	6	—0.10	0.00	+0.10	18.20	+2.10	0.71	+3.30	+3.30	+3.00	16.10	0.00	0.00
	9	—0.50	0.00	0.00	22.40	+6.30	2.14	+2.40	+2.70	+2.50	16.80	+0.70	0.24

is by no means universally accepted. *B. icteroides*, at one time regarded by Sanarelli as the causative organism of yellow fever, now regarded as a hog cholera bacillus, is another member of the group.

The hog cholera bacilli are usually found in swine, less commonly in man.

The organisms designated "Fowl cholera" were isolated from the diarrheal discharges of hens.

The distinctions between the various members of the paratyphoid group are somewhat vague: agglutination reactions do not always separate them sharply, because the group agglutinins are well marked in these organisms. Culturally they all produce gas in glucose and mannite, none in lactose and sucrose. Gelatin is not liquefied. Milk is acidified during the first few hours of incubation. Paratyphoid-β and the hog cholera bacilli produce, eventually, a strongly alkalin reaction in this medium after the glucose has been fermented, the medium eventually becoming almost opalescent due to the gradual solution of the casein. Paratyphoid-α is said to produce a permanent acidity in milk, resembling *B. typhosus* in this respect. The members of the paratyphoid group are not particularly active proteolytically, although on the average they are slightly more so than typhoid bacilli. They are intermediate between *B. typhosus* and *B. coli* in this respect.

The proteolytic activities of the hog cholera bacilli—Pierce Farm and Arkansas—deserve special mention. They were obtained from Prof. Theobald Smith. "Pierce Farm" is an avirulent culture, while "Arkansas" is very virulent. The "Pierce Farm" culture is much more active proteolytically than the "Arkansas" culture. This difference is partly due to the fact that the Pierce Farm culture grows more rapidly than the Arkansas culture.

Counts of the numbers of living bacteria at the end of 72 hours' incubation in the broths, in which these determinations in plain broth were made, gave the following results:

	Bacteria per cubic centimeter of plain broth.
Arkansas	*a* 8,700,000,000
	b 9,300,000,000
Pierce Farm "*a*"	*a* 12,000,000,000
	b 15,625,000,000

That is to say, there were about 64% as many "Arkansas" bacilli per cubic centimeter broth as there were "Pierce Farm" bacilli in the corresponding culture, yet the latter formed about four times as much ammonia during that time. This observation parallels one made previously by one of us (A. I. K.) in which the same difference in proteolytic activity was met with.[1]

[1] Kendall and Farmer, *J. Biol. Chem.*, **12**, 21 (1912).

Organism.	Days.	Plain broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.	Glucose broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.
Paratyphoid-α Arms	1	—0.40	+0.40	0.00	20.30	+ 0.70	0.21	+2.20	+2.90	+4.30	20.30	+0.70	0.21
	3	—0.60	+0.30	0.00	20.30	+ 1.00	0.30	+2.30	+4.10	+5.10	19.60	0.00	0.00
	6	—1.10	0.00	0.00	23.10	+ 3.50	1.04	+3.60	+4.00	+4.90	19.60	0.00	0.00
	9	—1.50	+0.20	—0.30	25.20	+ 5.60	1.67	+3.40	+4.10	+5.20	19.60	0.00	0.00
Paratyphoid-α P. D.	1	0.00	+0.20	+0.10	20.30	+ 0.70	0.21	+2.20	+3.30	+4.30	20.30	+0.70	0.21
	3	—0.80	+0.20	—0.10	21.70	+ 2.10	0.63	+2.20	+4.40	+5.40	19.60	0.00	0.00
	6	—1.00	—0.10	—0.10	24.50	+ 4.90	1.46	+3.80	+4.50	+5.40	19.60	0.00	0.00
	9	—1.50	—0.10	—0.50	28.70	+ 9.10	2.70	+3.60	+4.60	+5.80	19.60	0.00	0.00
Paratyphoid-α	1	+0.10	—0.10	+0.10	25.90	+ 1.40	0.41	+3.00	+3.00	+2.40	23.80	0.00	0.00
	3	—0.20	+0.30	—0.10	26.60	+ 2.10	0.61	+2.90	+4.10	+3.40	24.50	+0.70	0.22
	6	—1.70	+0.50	—0.20	30.10	+ 5.60	1.64	+3.00	+4.30	+3.60	25.90	+2.10	0.65
	9	—2.10	—0.30	+0.10	32.20	+ 7.70	2.24	+3.00	+4.00	+3.90	25.90	+2.10	0.65
Paratyphoid-β Arms	1	—0.30	0.00	—0.10	26.60	+ 2.10	0.61	+2.90	+2.60	+1.10	23.80	0.00	0.00
	3	—0.80	0.00	—0.20	28.00	+ 3.50	1.02	+3.70	+5.00	+4.00	24.50	+0.50	0.16
	6	—1.60	—0.20	—0.20	31.50	+ 7.00	2.04	+3.50	+4.60	+3.90	25.20	+1.40	0.44
	9	—1.60	—0.30	—0.40	37.80	+13.30	5.48	+2.40	+4.90	+4.40	25.90	+2.10	0.65
Paratyphoid-β P. D.	1	—0.30	+0.50	—0.20	20.30	+ 0.70	0.21	+2.80	+2.20	+4.10	20.30	+0.70	0.21
	3	—0.80	+0.40	+0.20	21.00	+ 1.40	0.42	+1.60	+3.10	+4.40	19.60	0.00	0.00
	6	—1.40	+0.20	0.00	24.50	+ 4.90	1.46	+1.50	+3.20	+4.20	19.60	0.00	0.00
	9	—2.60	+0.10	—0.40	28.00	+ 8.40	2.50	+2.30	+3.30	+4.50	19.60	0.00	0.00

Organism.	Days.	Plain broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	Per cent. NH_3/total N_2.	Glucose broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 Mg. per 100 cc. broth.	NH_3 Mg. increase per 100 cc. broth.	Per cent. NH_3/total N_2.
Paratyphoid-β	1	—0.60	0.00	0.00	28.70	+ 4.20	1.20	+3.30	+4.00	+2.90	25.90	+2.10	0.65
	3	—1.30	0.00	—0.30	30.10	+ 5.60	1.62	+3.10	+4.80	+3.70	25.20	+1.40	0.44
	6	—2.40	—0.20	—0.40	36.40	+11.90	3.47	+3.40	+4.70	+4.00	25.90	+2.10	0.65
	9	—2.40	—0.50	—0.60	39.90	+15.40	4.50	+3.80	+4.80	+3.90	26.60	+2.80	0.87
Paratyphoid-3	1	+0.30	—0.10	+0.10	25.20	+ 0.70	0.21	+3.20	+3.80	+2.80	24.50	+0.70	0.21
	3	—0.50	—0.20	—0.10	28.70	+ 4.20	1.25	+3.40	+3.40	+3.80	25.90	+2.10	0.63
	6	—0.60	0.00	+0.20	28.70	+ 4.20	1.25	+2.80	+3.80	+2.80	26.60	+2.80	0.83
	9	—0.80	0.00	—0.10	28.70	+ 4.20	1.25	+3.00	+3.70	+3.10	25.90	+2.10	0.63
Paratyphoid-1	1	—0.20	+0.20	+0.10	26.60	+ 0.70	0.19	+3.20	+3.30	+2.70	25.90	0.00	0.00
	3	—0.30	0.00	0.00	28.00	+ 2.10	0.58	+3.20	+3.80	+3.10	25.90	0.00	0.00
	6	—0.80	—0.10	—0.10	30.10	+ 4.20	1.15	+3.10	+3.80	+2.40	25.90	0.00	0.00
	9	—0.90	0.00	—0.10	32.20	+ 6.30	1.73	+3.00	+3.90	+2.70	25.90	0.00	0.00
Paratyphoid-2	1	+0.30	+0.20	+0.30	25.20	+ 0.70	0.20	+3.00	+3.50	+2.50	23.80	0.00	0.00
	3	—0.20	+0.20	+0.20	27.30	+ 2.80	0.82	+2.60	+3.30	+2.60	25.20	+1.40	0.44
	6	—1.50	+0.20	0.00	30.10	+ 5.60	1.64	+3.00	+4.00	+2.90	25.90	+2.10	0.65
	9	—0.70	—0.40	—0.30	34.30	+ 9.80	2.85	+3.30	+3.60	+3.40	26.60	+2.80	0.87
Paratyphoid-4	1	—0.30	0.00	0.00	26.60	+ 0.70	0.19	+3.20	+3.30	+4.40	25.90	0.00	0.00
	3	+0.10	0.00	+0.10	28.00	+ 2.10	0.58	+3.20	+3.70	+2.80	25.90	0.00	0.00
	6	—0.70	—0.70	0.00	29.40	+ 3.50	0.96	+3.10	+3.90	+2.70	25.90	0.00	0.00
	9	—1.20	+0.30	—0.40	31.50	+ 5.60	1.54	+3.00	+3.20	+2.70	26.60	+0.70	0.19

Paratyphoid-5	1	—0.10	—0.10	—0.10	26.60	+ 0.70	0.19	+3.00	+3.80	+2.40	25.20	—0.70	0.19
	3	—0.10	—0.50	0.00	27.30	+ 1.40	0.39	+3.20	+3.60	+3.10	25.20	—0.70	0.19
	6	—0.30	—0.20	—0.20	30.10	+ 4.20	1.15	+3.00	+3.50	+2.50	25.90	0.00	0.00
	9	—0.90	—0.10	—0.30	32.20	+ 6.30	1.73	+3.00	+3.40	+3.10	26.60	+0.70	0.19
Paratyphoid-6	1	—0.10	+0.20	0.00	26.60	+ 0.70	0.28	+3.10	+3.60	+2.80	25.20	—0.70	0.20
	3	+0.40	+0.20	+0.30	28.00	+ 2.10	0.62	+3.40	+3.50	+3.30	25.90	0.00	0.00
	6	—0.10	—0.30	+0.30	30.10	+ 4.20	1.25	+3.20	+3.70	+2.80	26.60	+0.70	0.20
	9	—0.90	0.00	+0.10	32.20	+ 6.30	1.87	+2.90	+3.70	+3.10	26.60	+0.70	0.20
Paratyphoid-7	1	—0.10	—1.00	+0.20	25.90	+ 1.40	0.41	+3.10	+3.50	+2.90	24.50	+0.70	0.22
	3	—1.30	0.00	—0.30	27.30	+ 2.80	0.82	+3.10	+4.80	+3.70	24.50	+0.70	0.22
	6	—0.80	+0.40	0.00	30.80	+ 6.30	1.84	+3.30	+4.30	+3.50	25.90	+2.10	0.65
	9	—1.30	—0.30	—0.10	34.30	+ 9.80	2.86	+3.40	+4.80	+3.90	25.90	+2.10	0.65
B. icteroides	1	+0.30	—0.10	+0.10	25.20	+ 0.70	0.21	+2.80	+3.40	+2.80	24.50	+0.70	0.21
	3	—0.50	—0.20	—0.10	28.70	+ 4.20	1.25	+3.00	+3.70	+2.80	25.90	+2.10	0.63
	6	—0.60	0.00	—0.20	28.70	+ 4.20	1.25	+3.40	+3.80	+3.10	26.60	+2.80	0.83
	9	—0.80	0.00	—0.50	28.70	+ 4.20	1.25	+3.20	+3.80	+3.80	25.90	+2.10	0.63
Hog cholera, Pierce farm	1	—0.40	—0.10	+0.10	20.30	+ 1.40	0.51	+2.80	+3.20	+3.00	18.20	—0.70	0.25
	3	—1.20	—0.40	—0.60	26.60	+ 7.70	2.82	+3.30	+4.20	+3.80	18.20	—0.70	0.25
	6	—2.20	—1.20	—0.90	31.50	+12.60	4.62	+3.10	+3.70	+3.50	18.90	0.00	0.00
	9	—2.20	—1.00	—0.90	35.00	+16.10	5.90	+3.10	+3.60	+3.40	19.60	+0.70	0.25
Hog cholera, Pierce farm "*a*"	1	—0.30	0.00	+0.10	20.30	+ 1.40	0.51	+2.90	+3.20	+3.00	18.90	0.00	0.00
	3	—1.80	—0.50	—0.70	28.70	+ 9.80	3.59	+3.30	+3.90	+3.60	18.90	0.00	0.00
	6	—1.90	—1.20	—0.90	30.80	+11.90	4.36	+3.20	+3.70	+3.60	18.90	0.00	0.00
	9	—2.70	—1.50	—1.50	35.70	+16.80	6.16	+3.10	+3.80	+3.50	20.30	+1.40	0.50
Hog cholera, Arkansas	1	—0.40	0.00	+0.10	19.60	+ 0.70	0.26	+1.60	+2.60	+2.10	18.20	—0.70	0.25
	3	—0.50	—0.30	0.00	21.70	+ 2.80	1.03	+2.70	+3.10	+2.80	18.20	—0.70	0.25
	6	—1.00	—0.10	—0.30	23.80	+ 4.90	1.79	+2.80	+3.10	+2.90	19.60	+0.70	0.25
	9	—0.70	—0.40	—0.30	25.90	+ 7.00	2.56	+2.80	+2.30	+2.50	19.60	+0.70	0.25

Organism.	Days.	Plain broth.						Glucose broth.					
		Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.	Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.
Hog cholera, Arkansas "A".	1	0.00	0.00	+0.20	15.40	+ 0.70	0.30	+2.30	+2.60	+2.90	14.70	+0.70	0.30
	3	—1.90	—1.60	—0.60	20.30	+ 5.60	2.42	+2.70	+3.10	+2.60	14.70	+0.70	0.30
	6	—1.80	—1.20	—0.90	21.70	+ 7.00	3.00	+2.70	+2.90	+2.80	14.70	+0.70	0.30
	9	—2.20	—1.20	—1.00	24.50	+ 9.80	4.25	+2.50	+2.80	+2.50	15.40	+1.40	0.61
Fowl cholera, I.....	1	—0.90	—0.10	+0.10	19.60	+ 0.70	0.26	+1.60	+2.00	+2.60	18.90	0.00	0.00
	3	—1.00	—0.10	—0.10	24.50	+ 4.90	1.72	+2.90	+3.30	+3.20	18.90	0.00	0.00
	6	—2.40	—0.70	—0.90	32.90	+14.00	5.12	+2.80	+3.20	+3.00	18.90	0.70	0.00
	9	—2.20	—1.40	—0.70	35.00	+16.10	5.90	+2.60	+3.30	+3.20	19.60	+0.00	0.25
Fowl cholera, II.....	1	—0.40	—0.10	+0.10	20.30	+ 1.40	0.51	+1.30	0.00	+2.30	18.90	0.00	0.00
	3	—0.70	—0.10	—0.90	21.70	+ 2.80	1.02	+3.10	+3.40	+3.30	18.90	0.00	0.00
	6	—1.60	—0.60	—1.10	26.60	+ 6.30	2.50	+2.90	+3.30	+3.20	18.90	0.00	0.00
	9	—1.80	—1.00	—1.30	30.10	+11.20	4.10	+2.80	+3.40	+3.20	19.60	+0.70	0.25

	Glucose.	Lactose.	Sucrose.	Mannite.	Milk.	Gelatin.
B. paratyphosus-α.....	*g*	—	—	*g*	+	—
B. paratyphosus-β.....	*g*	—	—	*g*	±	—
Morgan bacillus.....	*g*	—	—	—	±	—

XX. The Morgan Bacillus Group.

The organisms known as the Morgan bacillus were isolated originally by Dr. H. de R. Morgan of the Lister Institute of Preventive Medicine from the diarrheal discharges of children.[1] Its etiological relationship to certain types of summer diarrheas is not definitly known, but for the last three summers it has been found frequently and in relatively large numbers in certain of these cases seen in Boston. At times it appears to be present in dominant numbers, again it is associated with other bacteria, which are specifically known to be etiological factors in summer diarrheas, as for example, dysentery bacilli. It would be unjustifiable in the light of our present knowledge to dismiss this organism as a possible or even probable factor in the causation of intestinal disturbances milder than the average true bacillary dysenteries, which are conveniently classed as alimentary diarrheas. It is also found, not infrequently, in very mild types of disease. Its incidence in the feces of perfectly healthy children is not known, at least in this country.

Culturally it resembles *Bacillus paratyphosus-β* very closely except that it forms rather less gas in glucose and none in mannite. Inasmuch as mannite is a sugar, or rather, an alcohol not generally used in the cultural diagnosis of bacteria, this organism is at times diagnosed as a typical paratyphoid bacillus. Out of 18 cultures of paratyphoid collected from various sources, 3 have proved to be Morgan bacilli.

	Glucose.	Lactose.	Sucrose.	Mannite.	Milk.	Gelatin.	Indol.	Motility.
Morgan bacillus.....	*g*	—	—	—	±	—	—	+

Chemically the strains studied, which include one which Dr. Morgan sent one of us (A. I. K.), the remainder having been isolated from the diarrheal discharges of young children, are very different from paratyphoid bacilli. They are much more active proteolytically, producing relatively large amounts of ammonia in sugar-free broth. Even in glucose broth, a certain slight amount of proteolysis takes place, as is shown by the gradual accumulation of ammonia in it; proteolysis in sugar broth, however, neither proceeds as far nor as deeply as in broth free from sugar. The sparing action therefore of utilizable carbohydrate for protein is an important factor.

It is safe to say that the Morgan bacillus is not related very closely to the paratyphoid group, although culturally it resembles these organisms very closely It is worthy of note that the strains of this organism which have been identified culturally are very constant in their characteristics

[1] Morgan, *Brit. Med. J.*, 1907, April 6, 908–12; July 6, 16–19.

Organism.	Days.	Plain broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	Per NH_3/total N_2. cent.	Glucose broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	Per NH_3/total N_2. cent.
Morgan bacillus, I.....	1	+0.30	—0.30	0.00	28.00	+ 3.50	1.04	+3.00	+3.20	+2.20	28.00	+4.20	1.25
	3	—1.20	—0.10	—0.40	47.60	+23.10	6.88	+3.40	+4.60	+3.50	28.70	+4.90	1.46
	6	—1.80	—1.10	—0.70	56.00	+31.50	9.38	+2.80	+4.50	+3.40	30.10	+6.30	1.88
	9	—3.10	—0.90	—1.80	53.20	+27.80	8.55	+3.00	+4.40	+3.50	31.50	+7.70	2.29
Morgan bacillus, II.....	1	—0.90	—0.20	+0.10	46.90	+22.40	6.70	+2.80	+3.00	+2.20	28.00	+4.20	1.25
	3	—2.20	—0.30	—0.80	50.40	+25.90	7.70	+3.10	+4.30	+3.30	29.40	+5.60	1.67
	6	—4.70	—0.30	—1.20	53.20	+28.70	8.55	+2.60	+4.70	+3.30	30.10	+6.30	1.88
	9	—3.70	—2.00	—1.80	51.80	+27.30	8.14	+3.40	+4.60	+3.60	30.80	+7.00	2.08
Morgan bacillus, III.....	1	—0.50	—0.40	—0.20	29.40	+ 4.90	1.43	+1.30	+2.10	+1.50	28.70	+4.90	1.52
	3	—2.50	—0.70	—1.00	49.00	+24.50	7.14	+2.90	+3.50	+3.00	30.10	+6.30	1.96
	6	—3.70	—1.60	—1.30	58.10	+33.60	9.80	+2.60	+2.60	+1.20	32.90	+9.10	2.82
	9	—3.60	—2.10	—1.40	66.50	+42.00	12.25	+3.40	+3.60	+2.60	32.00	+8.40	2.60
Morgan bacillus, IV.....	1	—1.00	+0.10	+0.10	47.60	+23.10	6.74	+2.90	+3.30	+2.60	28.70	+4.90	1.52
	3	—2.40	+0.10	—0.50	49.70	+25.20	7.35	+3.20	+4.10	+3.40	30.10	+6.30	1.96
	6	—2.80	—0.60	—0.70	51.80	+27.30	7.96	+3.40	+4.40	+3.50	30.80	+7.00	2.17
	9	—5.30	—1.90	—1.90	61.60	+37.10	10.80	+2.90	+4.30	+3.60	31.50	+7.70	2.39
Morgan bacillus, V.....	1	—1.10	+0.10	+0.10	41.30	+15.40	4.59	+3.00	+3.10	+2.40	30.80	+4.90	1.43
	3	—1.20	—0.20	0.00	48.30	+22.40	6.68	+3.30	+3.10	+3.10	29.40	+3.50	1.02
	6	—1.20	—0.40	—0.60	49.00	+23.10	7.00	+3.10	+3.40	+2.90	30.10	+4.20	1.22
	9	—1.40	—0.50	—0.30	48.30	+22.40	7.95	+2.80	+3.60	+2.80	30.80	+4.90	1.43
Morgan bacillus, VI.....	1	—0.40	—0.30	+0.20	29.40	+14.70	6.38	+1.00	+2.30	+1.80	16.10	+2.10	0.91
	3	—1.00	—0.30	+0.10	31.50	+16.80	7.28	+2.30	+3.10	+2.70	17.50	+3.50	1.51
	6	—1.10	—0.60	—0.20	34.30	+19.60	8.50	+2.50	+2.90	+2.90	17.50	+3.50	1.51
	9	—1.50	—0.60	—0.10	35.00	+20.30	8.80	+2.40	+2.90	+2.80	18.20	+4.20	1.82

and the strains examined chemically are also very similar. This would be evidence in favor of the stability of the type.

XXI. B. coli Group.

The colon bacillus is an habitual parasite occurring in the intestinal tracts of man and the higher animals. Ordinarily it is a symbiote, adjusting its metabolism as the food presented to it in the alimentary canal varies, forming proteolytic products, such as ammonia, hydrogen sulfide, indole, skatol and phenols from protein or protein derivatives: carbon dioxide, hydrogen, lactic and smaller amounts of other lower fatty acids from carbohydrates. Its singular adaptability and plasticity in this respect probably explains its dominance among the intestinal bacteria.

B. coli is one of the relatively few bacteria which can utilize lactose, the animal sugar peculiarly characteristic of the mammalian milk, hence it would be predicted *a priori* that it would be found in the nursling intestinal flora, which is the case.

Proteolytically it is less active than *B. proteus*, a point of considerable significance in young babies. *B. proteus* does not ferment lactose. Rarely *B. coli* becomes invasive, but not progressively so in successive hosts, thus indicating that its offensive mechanism has not been perfected. Organisms like *B. typhosus* and *B. dysenteriae* on the contrary are less active proteolytically, but are as a rule invasive. Generally speaking, invasiveness is not compatible with well developed proteolytic powers.

	Glucose.	Lactose.	Sucrose.	Mannite.	Milk.	Gelatin.	Indol.	Motility.
B. coli (common type).....	*g*	*g*	—	*g*	*c*	—	+	+
B. coli (sucrose fermenting type)...	*g*	*g*	*g*	*g*	*c*	—	+	+

XXII. Bacillus Cloacae Group.

B. cloacae is an organism commonly found in sewage, but not as a rule found in the feces of man. It differs from *B. coli*, with which it is frequently confused, in several important details. Typically it liquefies gelatin but loses this property after long cultivation on ordinary media. Glucose broth is fermented, giving an inverted gas formula, that is, more carbon dioxide is formed than hydrogen in the proportion $H/CO_2 = 1/2$. This was first shown by Theobald Smith.[1] Members of the cloacae group decompose glucose rapidly. Even at the end of 24 hours this sugar has disappeared from the culture media as is shown by actual sugar determinations. The organisms, therefore, attack the protein, and this explains the large amount of ammonia produced in glucose broth after the first day. *B. coli*, under similar conditions, produces a far greater amount of

[1] Theobald Smith, "The Fermentation Tube," *Wilder Quarter Century Book*, Ithaca, **1893**, p. 212.

Organism.	Days.	Plain broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.	Glucose broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.
B. coli, I	1	—0.40	—0.20	0.00	25.20	+ 2.10	0.70	+3.80	+3.70	+3.10	23.10	0.00	0.00
	3	—1.00	—0.60	—0.40	31.50	+ 8.40	2.79	+4.40	+4.40	+3.80	21.70	—1.40	0.53
	6	—1.20	—0.60	—0.80	32.90	+ 9.80	3.25	+4.00	+4.40	+4.00	22.40	—0.70	0.26
	9	—1.60	—1.30	—1.00	34.30	+11.20	3.72	+3.80	+4.50	+4.00	21.70	—1.40	0.53
B. coli, I A	1	—0.30	0.00	0.00	28.00	+ 4.90	1.62	+3.80	+3.80	+3.50	23.10	0.00	0.00
	3	—0.90	—0.10	—0.30	30.10	+ 7.00	2.33	+4.00	+4.30	+3.80	21.70	—1.40	0.53
	6	—1.60	—1.00	—0.90	37.80	+14.70	4.88	+3.80	+4.30	+3.80	22.40	—0.70	0.26
	9	—1.70	—1.10	—1.20	39.20	+16.10	5.35	+4.20	+4.40	+4.10	21.70	—1.40	0.53
B. coli, I B	1	—1.30	+0.10	0.00	35.00	+10.50	2.83	+3.20	+4.30	+4.10	24.50	—0.70	0.19
	3	—2.00	0.00	—0.60	46.90	+21.50	5.80	+3.00	+5.20	+4.20	25.20	0.00	0.00
	6	—3.20	—0.20	—1.00	51.80	+27.30	7.36	+3.20	+5.40	+4.40	27.30	0.00	0.00
	9	—4.40	—0.60	—1.10	58.10	+33.60	9.10	+3.30	+5.60	+4.50	27.30	+2.10	0.57
B. coli, I C	1	—1.10	+0.10	+0.10	35.00	+10.50	2.83	+3.20	+4.30	+4.10	24.50	—0.70	0.19
	3	—2.00	0.00	—0.10	44.10	+19.60	5.28	+3.50	+5.30	+4.30	25.20	0.00	0.00
	6	—2.90	—0.20	—0.80	49.00	+24.50	6.70	+3.20	+5.60	+4.40	27.30	+2.10	0.57
	9	—3.00	—0.60	—0.90	52.50	+28.70	7.70	+3.90	+5.20	+4.60	27.30	+2.10	0.57
B. coli, Escherich	1	—0.90	0.00	—0.10	25.20	+ 7.00	2.44	+2.30	+2.90	+2.30	18.20	0.00	0.00
	3	—1.40	—0.60	—0.40	32.20	+14.00	4.88	+3.10	+2.90	+3.20	18.20	0.00	0.00
	6	—1.40	—1.00	—0.80	37.80	+19.60	6.84	+3.00	+3.80	+3.20	18.20	0.00	0.00
	9	—2.90	—1.00	—0.90	39.20	+21.00	7.32	+3.00	+4.10	+3.60	17.50	—0.70	0.19
B. coli, saccharose	1	—0.60	+0.20	—0.10	22.40	+ 4.20	1.46	+2.70	+3.00	+2.60	17.50	—0.70	0.24
	3	—1.20	—0.30	—0.40	32.90	+14.70	5.12	+2.50	+3.80	+2.90	18.20	0.00	0.00
	6	—1.60	—0.60	—0.40	40.60	+22.40	7.80	+2.90	+4.00	+3.10	18.20	0.00	0.00
	9	—1.80	—1.40	—1.00	39.90	+21.70	7.58	+2.80	+3.90	+3.00	18.20	0.00	0.00

B. coli, II	1	—0.90	—0.10	—0.30	27.30	+ 9.10	3.17	+2.50	+3.40	+2.60	16.80	—1.40	0.48
	3	—0.90	—0.60	—0.30	34.30	+16.10	5.64	+2.60	+3.70	+3.10	17.50	—0.70	0.24
	6	—1.60	—0.50	—0.40	42.00	+23.80	8.30	+2.80	+3.90	+3.20	18.20	0.00	0.00
	9	—1.80	—0.90	—0.80	40.60	+22.40	7.80	+2.90	+4.10	+3.20	18.20	0.00	0.00
B. coli, I D	1	—1.30	0.00	0.00	35.00	+10.50	2.83	+3.00	+4.30	+4.10	24.50	—0.70	0.19
	3	—2.00	—0.10	—1.00	46.90	+22.40	6.02	+3.20	+5.20	+4.30	25.20	0.00	0.00
	6	—3.20	—1.00	—1.10	51.80	+27.30	7.36	+3.30	+5.40	+4.40	27.30	+2.10	0.57
	9	—4.40	—1.10	—2.60	58.80	+34.30	9.23	+3.20	+5.60	+4.60	26.60	+1.40	0.38
B. coli, II B	1	—1.10	+0.20	+0.10	35.00	+10.50	2.83	+2.90	+4.30	+4.10	23.80	—1.40	0.38
	3	—2.00	0.00	—0.70	44.10	+19.60	5.24	+3.00	+5.30	+4.20	25.90	+0.70	0.19
	6	—2.90	—0.20	—0.40	49.00	+24.50	6.60	+3.20	+5.20	+4.40	27.30	+2.10	0.57
	9	—3.00	—0.60	—0.80	52.50	+28.00	7.55	+3.50	+5.60	+4.50	27.30	+2.10	0.57
B. coli, I E	1	—0.40	—0.10	—0.10	39.20	+10.30	1.18	+3.80	+4.30	+3.90	23.80	—0.70	0.19
	3	—2.60	—0.50	—0.60	51.80	+23.10	2.60	+4.00	+5.70	+4.60	23.80	—0.70	0.19
	6	—2.60	—0.40	—1.00	57.40	+28.70	3.23	+4.00	+5.90	+4.90	25.90	+1.40	0.38
	9	—3.10	—0.40	—1.40	65.10	+36.40	4.10	+4.70	+5.80	+5.00	25.90	+1.40	0.38
B. coli, I F	1	—0.30	—0.10	0.00	21.00	+ 4.20	1.54	+2.50	+2.90	+2.70	16.80	0.00	0.00
	3	—1.40	—0.10	—0.10	32.20	+15.40	5.64	+2.80	+2.80	+3.30	17.50	+0.70	0.25
	6	—1.80	—0.60	—0.60	38.50	+21.90	7.95	+3.00	+3.10	+3.50	17.50	+0.70	0.25
	9	—2.60	—0.90	—0.60	41.30	+24.50	8.99	+3.00	+4.10	+3.40	17.50	+0.70	0.25
B. coli, III	1	—0.60	+0.10	—0.30	21.70	+ 3.50	1.19	+2.90	+4.00	+5.10	18.20	0.00	0.00
	3	—2.20	—0.60	—0.90	35.70	+17.50	5.95	+3.50	+4.80	+6.00	18.20	0.00	0.00
	6	—3.00	—1.60	—1.30	37.10	+18.90	6.44	+4.90	+5.90	+6.90	18.20	0.00	0.00
	9	—2.20	—1.10	—1.40	38.50	+20.30	6.90	+3.90	+5.90	+7.00	18.20	0.00	0.00
B. coli, IV	1	—0.80	—0.10	—0.40	27.30	+ 9.10	3.10	+3.10	+4.10	+5.20	18.20	0.00	0.00
	3	—2.60	—0.70	—1.30	37.10	+18.90	6.44	+3.70	+4.40	+6.60	18.20	0.00	0.00
	6	—2.90	—2.20	—1.30	38.50	+20.30	6.90	+4.40	+5.70	+6.60	18.20	0.00	0.00
	9	—3.70	—1.90	—1.70	37.10	+18.90	6.44	+4.10	+6.10	+6.90	18.20	0.00	0.00

Organism.	Days.	Plain broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	Per cent. NH_3/total N_2.	Glucose broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	Per cent. NH_3/total N_2.
B. cloacae-3.....	1	—0.10	+0.10	+0.20	18.20	+ 1.40	0.51	+1.40	+1.90	+1.50	15.40	— 1.40	0.50
	3	—0.80	+0.10	—0.10	24.50	+ 7.70	2.82	+0.50	+1.60	+1.00	18.20	+ 1.40	0.50
	6	—2.10	—0.40	—0.60	35.00	+18.20	6.68	—0.40	+1.20	+0.50	24.50	+ 7.70	2.75
	9	—2.30	—0.90	—0.60	37.10	+20.30	7.44	—1.80	—0.60	—0.30	35.00	+18.20	6.50
B. cloacae-3a.....	1	—0.30	0.00	+0.10	19.60	+ 0.70	0.26	+1.10	+2.10	+2.00	17.50	— 1.40	0.50
	3	—1.20	—0.10	+0.70	26.60	+ 7.70	2.82	+0.40	+1.70	+1.20	22.40	+ 3.50	1.25
	6	—2.80	—1.20	+0.30	39.20	+20.30	7.44	—1.30	+0.50	—0.20	29.40	+10.50	3.75
	9	—2.80	—1.40	—0.50	40.60	+21.70	7.95	—2.00	—0.80	—0.10	38.50	+19.60	7.00
B. cloacae-2w.....	1	—0.40	—0.20	+0.10	19.60	+ 0.70	0.26	+0.70	+2.20	+1.80	16.80	— 2.10	0.75
	3	—2.00	—0.10	0.00	27.30	+ 8.40	3.08	+0.80	+1.40	+1.20	18.90	0.00	0.00
	6	—2.10	—1.40	—0.10	35.00	+16.10	5.90	—0.50	+0.90	+0.50	28.00	+ 9.10	3.25
	9	—2.90	—1.40	—0.50	39.90	+21.00	7.70	—1.20	+1.00	+0.50	35.70	+16.80	6.00

acid from glucose, checking its own growth thereby. This is an important permanent distinction between the colon group and the cloacae group.

	Glucose.	Lactose.	Sucrose.	Mannite.	Milk.	Gelatin.	Motility.	Indol.
B. cloacae.....	*g*	*g*	*g*	*g*	*c/p*	+	+	+

XXIII. Proteus Group.

Proteus bacilli were first isolated by Hauser[1] from putrefying meat infusions. Since that time they have been found to be widely distributed in nature, where their proteolytic activities are noteworthy in the bacterial degradation of albuminous substances to ammonia. Culturally, several varieties are known, but it is very probable that they are referable to a common type, *B. proteus vulgaris*. The observed differences are attributable to a partial loss of proteolytic or fermentative powers, a feature not uncommonly met with in bacteria of this type. The organisms differ typically from *B. coli* in two noteworthy respects—their inability to produce gas in lactose, and their great proteolytic powers.

	Glucose.	Lactose.	Sucrose.	Mannite.	Milk.	Gelatin.	Indol.	Motility.
B. proteus..	*g*	—	*g*	*g*	*c/p*	+	+	+

The proteus group is one of the most active chemically met with among the ordinary bacteria. The extent of the sparing action of glucose for protein is most marked in this group; about forty times as much ammonia is formed in sugar-free broth as is found under like conditions in glucose broth.

XXIV. Mucosus Capsulatus Group.

This group comprises various strains of organisms as follows: *Bacillus pneumonicum, B. rhinoscleroma, B. mucosus capsulatus, B. lactis aerogenes* and *B. acidi lactici*. The group is the poorest defined of those studied in this series, an observation in harmony with the experience of others. Culturally, the characteristic reactions common to all of the organisms include gas formation in glucose, lactose and mannite and the non-liquefaction of gelatin. A few strains produce gas in sucrose, some produce gas in polysaccharides, notably starch. Milk is coagulated by practically all members of this group, and certain strains form gas in it. A few cultures, notably strains of *B. mucosus capsulatus, lactis aerogenes* (from the University of Chicago), and *B. pneumonicum* of Pfeiffer and those designated "slimy", which were obtained by us from cases of severe intestinal diarrhea of babies, produce a viscous, mucin-like substance both in sugar-free and sugar broths. All of these organisms except the "slimy" forms use up the glucose at the end of about six days so that the ammonia production, which is minimal prior to this period, proceeds rapidly. The reaction rapidly becomes alkalin, neutralizing the acid formation during the initial fermentation period. In this group identity of cultural reaction

[1] Hauser, "Ueber Fäulniss Bakterien," Leipzig, 1885.

Organism.	Days.	Plain broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.	Glucose broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.
B. proteus vulgaris, I..	1	—0.40	+0.20	+0.20	19.60	+ 1.40	0.48	+1.40	+2.40	+1.80	18.20	0.00	0.00
	3	—1.00	—0.10	0.00	29.40	+11.20	3.90	+2.40	+3.30	+2.90	18.90	+0.70	0.24
	6	—1.40	—0.60	—0.20	52.50	+34.30	11.95	+2.70	+3.00	+2.80	20.30	+2.10	0.73
	9	—2.20	—1.20	—0.10	63.70	+45.50	15.85	+2.70	+3.30	+2.70	19.60	+1.40	0.48
B. proteus vulgaris, II.	1	—0.20	—0.10	+0.10	21.70	+ 2.80	1.02	+1.90	+2.80	+2.60	18.90	0.00	0.00
	3	—1.20	—0.40	+0.30	45.40	+26.60	9.72	+3.20	+3.80	+3.40	19.60	+0.70	0.25
	6	—3.10	—1.20	0.00	74.90	+54.20	19.80	+3.10	+3.60	+3.40	19.60	+0.70	0.25
	9	—3.40	—2.20	—0.80	83.30	+64.10	23.50	+2.80	+3.60	+3.40	20.30	+1.40	0.50
B. proteus vulgaris, III.	1	—0.10	0.00	0.00	21.00	+ 2.10	0.77	+1.80	+2.80	+2.70	19.60	+0.70	0.25
	3	—1.10	—0.30	+0.30	40.60	+21.70	7.95	+3.30	+3.80	+3.40	19.60	+0.70	0.25
	6	—3.30	—1.50	0.00	75.60	+56.70	20.75	+3.10	+3.60	+3.20	19.60	+0.70	0.25
	9	—4.30	—3.20	—1.30	80.50	+61.60	22.55	+2.90	+3.80	+3.40	20.30	+1.40	0.50

B. proteus vulgaris, IV.	1	+0.10	−0.20	+0.10	25.90	+ 2.80	0.89	+2.00	+2.30	+2.30	23.80	+0.70	0.23
	3	−1.00	−0.40	0.00	42.70	+19.60	6.22	+2.30	+2.80	+2.70	23.80	+0.70	0.23
	6	−2.60	−0.60	−0.10	57.40	+34.30	10.85	+2.20	+2.60	+2.50	24.50	+1.40	0.46
	9	−2.50	−0.90	−0.30	66.50	+43.40	13.80	+2.00	+2.80	+2.50	25.20	+2.10	0.68
B. proteus vulgaris, V.	1	−0.50	+0.10	0.00	31.50	+ 5.60	1.66	+3.20	+3.60	+2.00	27.30	+1.40	0.41
	3	+0.30	−0.50	+0.10	60.20	+34.30	14.50	+3.30	+3.40	+3.30	26.60	+0.70	0.20
	6	−3.80	−0.70	−0.80	86.80	+60.90	18.10	+3.11	+3.60	+3.00	27.30	+1.40	0.41
	9	−4.10	−1.80	−0.80	90.30	+64.40	19.20	+4.30	+3.50	+3.00	27.30	+1.40	0.41
B. proteus vulgaris, VI.	1	−0.70	−0.30	0.00	29.40	+10.50	3.85	+2.20	+3.00	+2.90	18.20	−0.70	0.25
	3	−1.60	−0.60	+0.30	42.00	+23.10	8.48	+5.60	+4.20	+3.90	18.90	0.00	0.00
	6	−0.80	−1.20	+0.10	77.00	+58.10	21.30	+3.10	+4.30	+3.80	18.90	0.00	0.00
	9	−4.00	−2.40	−0.70	92.40	+73.50	26.90	+3.30	+4.10	+3.70	18.60	+0.70	0.25
B. proteus vulgaris, *a*.	1	−0.10	−0.10	+0.10	21.70	+ 2.80	1.02	+1.90	+3.00	+2.60	19.60	+0.70	0.25
	3	−1.10	0.00	−0.10	40.60	+21.70	7.95	+3.10	+3.60	+3.10	19.60	+0.70	0.25
	6	−2.50	0.00	0.00	68.60	+49.70	18.20	+2.90	+3.30	+3.20	19.60	+0.70	0.25
	9	−5.00	−3.20	−0.80	77.00	+58.10	21.30	+3.00	+3.80	+3.30	20.30	+1.40	0.50
B. proteus vulgaris, IIa.	1	−0.30	+0.10	+0.10	19.60	+ 2.80	1.02	+2.60	+3.00	+2.60	16.80	0.00	0.00
	3	+0.40	0.00	0.00	29.40	+12.60	4.62	+2.80	+3.50	+2.80	18.20	+1.40	0.50
	6	−1.80	−0.20	−0.10	52.50	+35.40	12.97	+2.70	+3.30	+3.00	18.20	+1.40	0.50
	9	−2.60	−0.90	−0.20	73.50	+56.30	20.60	+3.00	+3.50	+3.00	18.20	+1.40	0.50

Organism.	Days.	Plain broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	Per NH_3/total N_2. cent.	Glucose broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	Per NH_3/total N_2. cent.
B. lactis aerogenes-1.....	1	—0.20	+0.30	+0.20	20.30	+ 0.70	0.21	+3.60	+3.20	+3.00	19.60	0.00	0.00
	3	—0.70	—0.10	—0.10	20.30	+ 0.70	0.21	+3.90	+4.40	+3.80	19.60	0.00	0.00
	6	—2.00	—0.40	—0.60	23.80	+ 4.20	1.25	+3.90	+4.60	+4.10	19.60	0.00	0.00
	9	—2.30	—0.80	—0.90	28.70	+ 4.20	1.25	+4.20	+5.00	+4.40	19.60	0.00	0.00
B. lactis aerogenes-2.....	1	—0.20	+0.20	+0.20	20.30	+ 0.70	0.21	+3.80	+3.40	+3.20	19.60	0.00	0.00
	3	—1.00	0.00	—0.30	21.00	+ 1.40	0.42	+4.00	+4.40	+3.90	19.60	0.00	0.00
	6	—2.00	—0.30	—0.50	24.50	+ 4.90	1.46	+4.00	+4.70	+4.30	18.90	—0.70	0.21
	9	—2.10	—0.70	—0.90	27.30	+ 7.70	2.29	+4.20	+5.00	+4.30	19.60	0.00	0.00
B. lactis aerogenes-3.....	1	—0.60	+0.30	0.00	20.30	+ 0.70	0.21	+3.60	+3.50	+3.20	20.30	+0.70	0.21
	3	—0.60	0.00	—0.30	20.30	+ 0.70	0.21	+4.50	+4.50	+4.30	19.60	0.00	0.00
	6	—2.10	—0.90	—0.90	25.90	+ 6.30	1.87	+4.30	+5.00	+4.30	19.60	0.00	0.00
	9	—2.60	—0.70	—1.00	27.30	+ 7.70	2.29	+4.10	+5.00	+4.20	19.60	0.00	0.00
B. lactis aerogenes-4.....	1	—0.90	+0.20	—0.20	20.30	+ 0.70	0.21	+3.50	+3.60	+3.20	19.60	0.00	0.00
	3	—1.20	—0.10	—0.30	21.00	+ 1.40	0.42	+3.80	+4.60	+4.00	19.60	0.00	0.00
	6	—2.10	—0.20	—1.10	24.50	+ 4.90	1.46	+4.40	+5.10	+4.30	19.60	0.00	0.00
	9	—2.60	—0.70	—1.10	28.00	+ 8 40	2.50	+3.90	+5.10	+4.40	19.60	0.00	0.00
B. lactis aerogenes-5.....	1	—0.30	+0.40	0.00	20.30	+ 0.70	0.21	+3.40	+3.20	+3.20	20.30	+0.70	0.21
	3	—1.00	+0.30	—0.20	21.00	+ 1.40	0.42	+4.20	+4.40	+4.00	19.60	0.00	0.00
	6	—1.60	—0.10	—0.20	22.40	+ 2.80	0.83	+4.20	+4.90	+4.30	19.60	0.00	0.00
	9	—2.50	—0.55	—1.00	25.20	+ 5.60	1.67	+4.30	+4.50	+4.10	19.60	0.00	0.00
Slimy, 6*d*, I.....	1	—1.00	0.00	0.00	27.30	+ 1.40	0.42	+3.10	+3.60	+2.80	25.20	—0.70	0.21
	3	—1.20	—0.90	—0.40	32.90	+ 7.40	2.20	+4.30	+2.90	+3.30	25.90	0.00	0.00
	6	—1.20	—1.10	—0.80	34.30	+ 8.40	2.50	+2.90	+4.80	+3.40	26.60	+0.70	0.21
	9	—1.50	—1.10	—1.40	39.90	+14.00	5.94	+3.20	+4.40	+3.50	27.30	+1.40	0.41

Slimy, *6d*, II	1	—0.50	0.00	—0.20	27.30	+ 1.40	0.42	+3.20	+4.00	+3.10	25.90	0.00	0.00
	3	—0.90	—0.70	—0.20	27.30	+ 1.40	0.42	+3.90	+4.20	+4.00	25.20	—0.70	0.20
	6	—1.90	—2.60	—1.10	34.30	+ 8.40	2.50	+3.20	+4.70	+3.40	26.60	+0.70	0.20
	9	—1.70	—1.20	—1.40	37.80	+11.90	3.54	+3.10	+4.20	+3.50	27.30	+1.40	0.41
Slimy, 53	1	—0.70	—0.40	—0.50	27.30	+ 1.40	0.39	+3.00	+4.20	+3.40	25.90	0.00	0.00
	3	—0.50	—0.30	—0.70	29.40	+ 3.50	0.96	+3.70	+4.10	+3.60	25.90	0.00	0.00
	6	—1.00	—0.50	—0.80	32.20	+ 6.30	1.73	+3.20	+4.50	+3.30	25.90	0.00	0.00
	9	—1.60	—0.60	—0.30	37.10	+11.20	3.15	+3.20	+4.30	+3.70	27.30	+1.40	0.41
Slimy, 53*d*	1	—0.50	—0.20	—0.50	27.30	+ 1.40	0.42	+4.60	+4.20	+3.60	25.90	0.00	0.00
	3	—0.70	—0.30	—0.40	28.00	+ 2.10	0.58	+3.50	+4.00	+3.60	25.90	0.00	0.00
	6	—1.10	—0.50	—0.60	32.90	+ 7.00	1.92	+3.50	+4.60	+3.60	25.90	0.00	0.00
	9	—1.40	—0.60	—0.50	36.40	+10.50	2.89	+3.30	+4.30	+3.80	27.30	+1.40	0.41
B. mucosus capsulatus, I	1	—0.10	0.00	0.00	19.60	+ 0.70	0.27	+1.80	+2.50	+2.30	18.20	—0.70	0.26
	3	—0.80	—0.20	0.00	21.00	+ 2.10	0.81	+0.90	+1.50	+1.10	19.60	+0.70	0.26
	6	—1.10	—0.50	—0.20	28.70	+ 9.80	3.78	+0.70	+0.30	+0.40	20.30	+1.40	0.51
	9	—1.30	—0.20	+0.10	33.60	+14.70	5.70	+0.50	+0.10	+0.40	24.50	+5.60	2.05
B. mucosus capsulatus, II	1	—0.20	0.00	0.00	19.60	+ 0.70	0.27	+1.60	+2.50	+2.30	17.50	—1.40	—0.51
	3	—0.40	—0.20	+0.20	21.00	+ 2.10	0.81	+0.30	+2.60	+1.00	18.90	0.00	0.00
	6	—0.70	—0.20	—0.30	26.60	+ 7.70	2.97	+1.80	+2.50	+1.20	21.70	+2.80	1.03
	9	—0.80	—0.20	—0.10	32.20	+13.30	5.14	+1.20	+1.40	+1.10	27.30	+8.40	3.08
B. mucosus capsulatus, III	1	0.00	0.00	0.00	19.60	+ 0.70	0.27	+1.60	+2.60	+2.30	17.50	—1.40	0.51
	3	—0.40	—0.20	+0.10	21.00	+ 2.10	0.81	+0.50	+1.40	+0.90	18.90	0.00	0.00
	6	—0.50	—0.20	0.00	25.90	+ 7.00	2.70	+0.70	+1.40	+0.80	21.10	+2.20	0.81
	9	—1.10	—0.30	—0.10	33.60	+14.70	5.58	+0.70	+1.20	+1.00	25.20	+6.30	2.30
B. mucosus capsulatus, IV	1	—0.40	0.00	0.00	19.60	+ 0.70	0.27	+1.90	+2.40	+2.20	18.20	—0.70	0.26
	3	—0.80	—0.20	—0.20	21.70	+ 2.80	1.08	+1.00	+1.30	+1.00	18.90	0.00	0.00
	6	—0.50	—0.20	—0.30	26.60	+ 7.70	2.97	+0.30	+1.50	+0.70	20.30	+1.40	0.51
	9	—0.70	—0.20	—0.10	34.30	+15.40	5.94	+0.40	+1.50	+1.10	25.20	+6.30	2.30

Organism.	Days.	Plain broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	Per NH_3/total N_2. cent.	Glucose broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	Per NH_3/total N_2. cent.
B. mucosus capsulatus, V	1	—0.10	0.00	0.00	19.60	+ 0.70	0.27	+1.20	+2.80	+1.20	17.50	—1.40	0.51
	3	—0.30	0.00	—0.20	21.70	+ 2.80	1.08	+0.70	+1.20	+0.90	18.90	0.00	0.00
	6	—0.40	—0.30	—0.30	26.60	+ 7.70	2.97	+0.40	+1.90	+1.10	21.10	+2.20	0.81
	9	—0.80	—0.20	—0.10	34.30	+15.40	5.94	+0.40	+1.50	+1.00	23.10	+4.20	1.54
B. mucosus capsulatus, VI	1	—0.10	+0.10	+0.10	19.60	+ 0.70	0.27	+1.80	+2.30	+2.40	18.20	—0.70	0.26
	3	—0.60	0.00	0.00	21.00	+ 2.10	0.81	+0.60	+1.50	+0.90	18.90	0.00	0.00
	6	—0.40	0.00	—0.10	25.90	+ 7.00	2.70	+0.60	+1.20	+0.70	21.70	+2.80	1.03
	9	—1.30	—0.70	—0.30	37.10	+18.20	7.02	+0.70	+1.30	+0.90	26.60	+7.70	2.84
B. mucosus capsulatus, VII	1	0.00	+0.10	+0.10	19.60	+ 0.70	0.27	+1.70	+2.10	+2.20	17.50	—1.40	0.51
	3	—0.40	+0.10	0.00	21.70	+ 2.80	1.08	+0.90	+1.20	+0.90	18.90	0.00	0.00
	6	—0.70	0.00	0.00	28.70	+ 9.80	3.78	+1.40	+1.30	+1.00	19.60	+0.70	0.26
	9	—0.80	—0.20	—0.20	30.80	+11.90	4.60	+1.10	+1.70	+1.20	24.50	+5.60	2.05
B. mucosus capsulatus, VIII	1	—0.40	+0.10	+0.10	25.20	+ 2.10	0.67	+1.90	+2.10	+2.00	23.10	0.00	0.00
	3	—0.40	—0.20	0.00	27.30	+ 4.20	1.34	+1.40	+1.90	+1.50	23.80	+0.70	0.23
	6	—3.30	—2.10	—0.90	47.60	+24.50	7.78	+0.60	+1.10	+0.80	26.60	+3.50	1.13
	9	—2.50	—1.10	—0.50	47.60	+24.50	7.78	—0.80	0.00	+0.20	31.15	+8.05	2.60
B. mucosus capsulatus, Pfeiffer	1	0.00	+0.20	—0.10	25.90	+ 2.80	0.89	+1.20	+1.30	+1.20	23.10	0.00	0.00
	3	—2.00	—0.60	—0.60	35.70	+12.60	4.00	+0.30	+1.40	+1.30	23.80	+0.70	0.23
	6	—3.80	—1.80	—1.60	50.40	+27.30	8.70	+0.10	+0.70	+0.40	26.60	+3.50	1.13
	9	—3.80	—3.10	—1.90	50.40	+27.30	8.70	—0.60	+0.40	+0.30	34.30	+11.20	3.64

qualitatively, does not necessarily correspond to chemical activity quantitatively. A comparison of the cultural characteristics and chemical reaction brings this out clearly. The important cultural characteristics appear in the following chart:

	Glucose.	Lactose.	Sucrose.	Mannite.	Milk.	Gelatin.	Indol.	Starch.	Mucus.
B. mucosus capsulatus, I.....	*g*	*g*	*g*	*g*	*c/g*	—	+	*g*	+
B. mucosus, II–VII.....	*g*	*g*	*g*	*g*	*c/g*	—	—	*g*	+
B. mucosus, Pfeiffer.....	*g*	*g*	*g*	*g*	*c/g*	—	—	*g*	+
B. lactis aerogenes (Chicago).....	*g*	*g*	*g*	*g*	*c/g*	—	—	*g*	+
B. lactis aerogenes –IV.....	*g*	*g*	—	*g*	*c/g*	—	+	—	—
Slimy 6*d*–6*d*, I.....	*g*	*g*	—	*g*	*c/g*	—	—	—	+
Slimy 53*d*–53*d*, I.....	*g*	*g*	—	*g*	*c*	—	+	—	+

In view of the confusion found in the identification of various members belonging to the *Mucosus Capsulatus* group of bacteria it is impossible to state here the specific names which the various members should bear. Perkins[1] has formulated what appears to be the simplest and best description of this group. According to his nomenclatures cultures designated as *mucosus capsulatus*, I to VII, *lactis aerogenes*, Chicago, and *B. mucosus*, Pfeiffer, would be regarded as *B. lactis aerogenes: B. lactis aerogenes*, I to V, become *B. acidi lactici*. Slimy bacillus 6*d* and 53*d* would be regarded as variants of the *lactis aerogenes* group both chemically and in virtue of their mucus formation. From the bacterial point of view these strains of bacteria are rather uncertain: some of them are not identified culturally with any known members of this group. Chemically, the results which appear in the following tables are suggestive, indicating as they do that the bacterial identity of cultural characteristics does not necessarily imply identity of species. Further work with large numbers of strains will be necessary to establish the *mucosus capsulatus-lactis aerogenes-acidi lactici* group on a logical basis. It should be stated that none of these organisms culturally belong to the colon group.

XXV. B. cuniculicida.

The series of organisms designated as *B. cuniculicida* are of unknown origin. They are not members of the true hemorrhagic septicemia group specifically pathogenic for rabbits, which are customarily (see study XVIII) designated by this specific name. Culturally they are practically identical with the Flexner bacillus except that they form indol as is indicated in the chart.

	Glucose.	Lactose.	Sucrose.	Mannite.	Milk.	Gelatin.	Indol.
B. cuniculicida.....	+	—	—	+	+	—	+
B. dysenteriae (Flexner)..	+	—	—	+	+	—	—

Chemically *B. cuniculicida* is much more active than the Flexner bacillus. There is no apparent relation between the two groups.

[1] Perkins, *J. Inf. Diseases*, 1, 241–67 (1904).

Organ sm.	Days.	Plain broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.	Glucose broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.
B. cuniculicida-1.....	1	+0.20	+1.00	+0.60	24.50	+ 4.90	1.46	+1.90	+2.70	+2.20	23.80	+4.20	1.22
	3	—0.66	+0.40	+0.30	29.40	+ 9.80	2.91	+1.60	+2.60	+2.20	23.80	+4.20	1.22
	6	—1.00	+0.30	0.00	34.30	+14.70	4.08	+1.60	+2.90	+2.70	23.80	+4.20	1.22
	9	—1.80	—0.10	—0.30	39.90	+20.30	6.02	+1.90	+3.40	+2.40	25.20	+5.60	1.63
B. cuniculicida-2.....	1	+0.30	+1.10	+0.80	24.50	+ 4.90	1.46	+1.70	+2.50	+2.40	23.10	+3.50	1.02
	3	—0.60	+0.40	+0.30	30.80	+11.20	3.33	+1.40	+2.30	+2.20	23.80	+4.20	1.22
	6	—1.10	+0.30	0.00	35.70	+16.10	4.80	+1.20	+3.40	+2.50	22.40	+2.80	0.82
	9	—1.30	0.00	0.00	40.60	+21.00	6.25	+2.10	+3.60	+2.90	24.50	+4.90	1.43
B. cuniculicida-3.....	1	0.00	+1.00	+0.60	23.80	+ 4.20	1.25	+1.80	+1.90	+2.00	23.80	+4.20	1.22
	3	—0.20	+1.00	+0.50	28.70	+ 9.10	2.71	+1.20	+2.10	+2.30	23.80	+4.20	1.22
	6	—1.30	+0.10	+0.10	35.70	+16.10	4.80	+0.80	+3.00	+2.60	23.80	+4.20	1.22
	9	—1.40	+0.10	—0.10	39.90	+20.30	6.02	+1.90	+3.80	+3.30	23.80	+4.20	1.22
B. cuniculicida-4.....	1	+0.20	+1.00	+0.60	22.40	+ 2.80	0.83	+1.80	+2.00	+2.00	23.10	+3.50	1.02
	3	—1.00	+0.40	+0.20	27.30	+ 7.70	2.30	+0.80	+2.00	+2.30	23.80	+4.20	1.22
	6	—1.00	+0.40	—0.20	29.40	+ 9.80	2.91	+1.70	+3.40	+3.10	23.80	+4.20	1.22
	9	—1.60	+0.20	—0.30	36.40	+16.80	5.00	+2.10	+3.60	+2.80	23.80	+4.20	1.22

B. cuniculicida-4a.....	1	—0.10	0.00	0.00	18.90	+ 2.80	0.95	+0.80	+1.80	+1.50	17.50	+1.40	0.48
	3	+0.30	+0.60	+0.50	21.70	+ 5.60	1.90	+1.40	+2.20	+2.00	18.20	+2.10	0.71
	6	+0.30	+0.40	+0.40	31.50	+15.00	5.24	+0.90	+1.90	+1.70	17.50	+1.40	0.48
	9	—0.70	—0.30	+0.20	37.80	+21.70	7.40	+0.90	+1.90	+1.70	18.20	+2.10	0.71
B. cuniculicida-4b.....	1	+0.30	0.00	0.00	18.20	+ 2.10	0.71	+0.70	+1.60	+1.20	17.50	+1.40	0.48
	3	+0.30	+0.70	+0.60	20.30	+ 4.20	1.43	+1.30	+2.00	+1.90	17.50	+1.40	0.48
	6	+0.20	—0.10	+0.60	23.10	+ 7.00	2.38	+1.40	+1.80	+1.70	17.50	+1.40	0.48
	9	—0.70	—0.20	+0.30	37.80	+21.70	7.40	+1.00	+2.10	+1.90	18.20	+2.10	0.71
B. cuniculicida-4c.....	1	0.00	0.00	0.00	18.20	+ 2.10	0.71	+0.80	+2.00	+1.80	17.50	+1.40	0.48
	3	+0.30	+0.80	+0.60	21.70	+ 5.60	1.90	+1.20	+2.00	+1.90	18.20	+2.10	0.71
	6	—0.80	—0.20	+0.10	31.50	+15.40	5.24	+1.10	+1.90	+1.80	18.20	+2.10	0.71
	9	—0.80	—0.80	0.00	37.10	+21.00	7.10	+1.10	+2.10	+2.00	18.20	+2.10	0.71
B. cuniculicida-4d.....	1	0.00	0.00	+0.10	18.90	+ 2.80	0.95	+0.90	+2.00	+1.90	17.50	+1.40	0.48
	3	+0.40	+0.30	+0.80	21.70	+ 5.60	1.90	+1.60	+2.20	+1.90	18.20	+2.10	0.71
	6	0.00	+0.50	+0.50	26.60	+10.50	3.57	+1.10	+2.00	+2.00	17.50	+1.40	0.48
	9	—0.50	0.00	+0.30	34.30	+18.20	6.20	+1.10	+2.30	+2.20	18.20	+2.10	0.71
B. cuniculicida-4e.....	1	—0.10	0.00	+0.10	18.20	+ 2.10	0.71	+0.70	+1.70	+1.50	17.50	+1.40	0.48
	3	—0.10	+0.40	+0.40	23.80	+ 7.70	2.62	+1.40	+2.00	+1.90	18.20	+2.10	0.71
	6	—0.20	0.00	+0.50	28.00	+11.90	4.05	+0.90	+1.80	+1.90	18.20	+2.10	0.71
	9	—0.90	—0.40	+0.10	39.20	+23.10	7.86	+1.10	+2.00	+2.10	18.20	+2.10	0.71

Organism.	Days.	Plain broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.	Glucose broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.
Sp. cholerae, Hopkins..	1	—0.30	0.00	+0.20	18.90	0.00	0.00	+1.80	+1.10	+1.30	18.20	—0.70	0.25
	3	—0.50	—0.10	—0.10	25.90	+ 2.00	1.75	+1.90	+2.10	+1.90	18.90	0.00	0.00
	6	—1.00	—0.40	—0.40	38.50	+19.60	4.93	+2.30	+2.20	+2.10	19.60	+0.70	0.25
	9	—2.00	—1.20	—1.20	54.60	+35.70	8.95	+2.20	+2.00	+1.80	18.90	0.00	0.00
Sp. cholerae, P. I.....	1	—0.10	—0.20	0.00	19.60	+ 0.70	0.18	+1.80	+2.10	+1.70	18.90	0.00	0.00
	3	—1.10	—0.50	—0.80	31.50	+12.60	3.16	+1.70	+2.10	+1.70	18.90	0.00	0.00
	6	—1.80	—1.00	—1.20	56.00	+37.10	9.30	+2.00	+1.90	+1.40	18.90	0.00	0.00
	9	—3.00	—1.40	—1.40	62.30	+43.40	10.80	+1.60	+2.10	+1.70	19.60	+0.70	0.25
Sp. cholerae, Berlin...	1	—0.20	0.00	+0.10	20.30	+ 1.40	0.33	+1.20	+1.60	+0.50	18.90	0.00	0.00
	3	—1.20	—1.60	—0.10	44.10	+25.20	6.31	+1.50	+2.00	+1.60	19.60	+0.70	0.25
	6	—2.90	—1.20	—0.90	60.20	+41.30	10.35	+1.50	+2.00	+1.60	19.60	+0.70	0.25
	9	—3.00	—1.60	—1.40	67.20	+48.30	12.10	+1.80	+1.80	+1.60	19.60	+0.70	0.25
Sp. cholerae, Hamburg	1	—0.30	—0.10	+0.20	21.00	+ 2.10	0.53	+1.70	+1.80	+1.40	19.60	+0.70	0.25
	3	—1.80	—0.80	—0.10	48.30	+29.40	7.39	+1.50	+2.00	+1.30	19.60	+0.70	0.25
	6	—3.10	—1.60	—0.10	67.90	+49.00	12.30	+2.00	+2.30	+1.80	19.60	+0.70	0.25
	9	—3.00	—1.60	—1.50	67.20	+48.30	12.10	+1.60	+2.10	+1.70	20.30	+1.40	0.50
Sp. cholerae, Boston, *a*	1	0.00	+0.10	0.00	19.60	+ 0.70	0.18	+1.40	+1.40	+1.10	18.20	—0.70	0.25
	3	+0.30	+0.20	+0.20	21.00	+ 2.10	0.53	+2.40	+3.30	+3.00	18.90	0.00	0.00
	6	+0.30	+0.10	0.00	23.10	+ 4.20	1.05	+2.00	+3.50	+3.00	18.90	0.00	0.00
	9	—0.30	—0.40	—0.20	26.60	+ 7.70	1.93	+2.00	+3.40	+2.90	19.60	+0.70	0.25

Sp. cholerae, Boston, *b.*	1	—0.10	0.00	+0.10	16.10	+ 0.70	0.25	+3.00	+3.20	+3.00	16.10	0.00	0.00
	3	—0.30	+0.10	+0.20	16.10	+ 0.70	0.25	+3.40	+3.90	+3.80	16.10	0.00	0.00
	6	—0.40	0.00	+0.20	19.60	+ 4.20	1.50	+3.40	+4.30	+3.50	16.10	0.00	0.00
	9	—0.50	—0.10	0.00	22.30	+ 6.90	0.00	+3.50	+4.40	+3.70	16.80	+0.70	0.22
Vibrio Nassac.....	1	0.00	+0.10	—0.10	18.20	0.00	0.00	—0.70	+1.90	+1.30	18.20	0.00	0.00
	3	—1.60	0.00	—0.10	35.70	+17.50	6.10	0.00	+2.00	+1.30	18.20	0.00	0.00
	6	—3.70	—2.30	—1.50	53.90	+35.70	12.40	+0.60	+1.80	+1.50	18.20	0.00	0.00
	9	—3.90	—2.50	—1.80	65.80	+47.60	16.60	+0.70	+2.00	+1.10	18.20	0.00	0.00
Sp. Finkler and Prior..	1	0.00	0.00	+0.20	18.20	+ 1.40	0.51	+1.20	+2.00	+1.70	16.80	0.00	0.00
	3	—0.10	—0.10	—0.30	28.00	+11.20	4.10	+1.50	+2.20	+1.80	16.80	0.00	0.00
	6	—1.40	—0.40	—0.50	35.70	+18.90	6.93	+1.00	+2.10	+1.70	17.50	+0.70	0.25
	9	—1.80	—1.00	—0.60	44.10	+27.30	10.00	+1.40	+1.50	+2.00	17.50	+0.70	0.25
Sp. Metchnikovi.....	1	—0.20	—0.30	—0.10	25.90	+ 2.80	0.89	+2.50	+2.70	+2.50	23.10	0.00	0.00
	3	—2.10	—1.10	—0.50	41.30	+18.20	5.80	+1.60	+2.40	+2.20	23.10	0.00	0.00
	6	—3.60	—2.40	—0.60	64.40	+41.30	13.10	+2.40	+2.90	+3.00	23.10	0.00	0.00
	9	—4.10	—4.30	—1.70	64.40	+41.30	13.10	+2.10	+2.70	+2.30	23.80	+0.70	0.23
Vibrio H/61.....	1	—0.10	+0.10	0.00	17.50	—1.70	0.24	—0.60	0.00	—0.30	18.20	0.00	0.00
	3	—0.60	—0.50	—0.80	16.80	—1.40	0.49	—1.40	—0.50	—1.20	18.20	0.00	0.00
	6	—1.00	—1.30	—1.00	16.80	—1.40	0.49	—1.90	—1.20	—1.50	18.20	0.00	0.00
	9	—1.80	—0.70	—1.30	16.80	—1.40	0.49	—2.70	—0.80	—1.80	19.60	+1.40	0.49
Vibrio H/120.....	1	—0.20	0.00	—0.10	17.50	—0.70	0.24	—0.80	—0.10	—0.20	16.80	—1.40	0.49
	3	—0.90	—0.70	—1.00	16.80	—1.40	0.49	—1.70	—1.00	—1.40	18.90	+0.70	0.24
	6	—1.40	—0.70	—1.00	16.80	—1.40	0.49	—2.30	—1.40	—1.50	21.00	+2.80	0.98
	9	—0.80	—0.40	—0.90	16.80	—1.40	0.49	—2.10	—1.00	—1.80	20.30	+2.10	0.75
Vibrio H/120a.....	1	—0.40	—0.20	—0.20	15.40	0.00	0.00	—0.60	—0.20	—0.50	15.40	—0.70	0.22
	3	—1.30	—0.30	—0.50	14.70	—0.70	0.25	—0.90	—0.20	—0.60	11.20	—4.90	1.52
	6	—1.90	—0.80	—0.80	14.70	—0.70	0.25	—2.00	—0.80	—1.00	11.20	—4.90	1.52
	9	—2.70	—1.60	—1.10	16.80	+1.40	0.50	—2.70	—1.60	—1.50	11.90	—4.20	1.30

XXVI. Vibrio Group.

Asiatic cholera is one of the most dreaded diseases of man. Clinically the disease is characterized by a very short incubation period, quite unlike typhoid fever, for example. A man may be exposed to infection and die of it within 18 hours, although typically the incubation period is longer. Culturally and chemically the cholera vibrios are as unlike the organisms causing toxemias, as the clinical disease cholera is unlike typhoid fever, for example. Cholera vibrios are very active proteolytically, resembling proteus bacilli grossly in this respect.

Spirillum cholerae, Sp. of Finkler and Prior, *Sp. Metchnikovi* and *Vibrio Nassac* are very similar culturally and chemically. They all utilize the common sugars, forming acid, but no gas. They form acid in milk and liquefy gelatin.

Vibrios H/61 and *H/120* were isolated by Dr. Arms, of the Boston Board of Health Laboratory, from the feces of suspected cholera patients. These organisms are very unlike cholera chemically; they utilize no sugars, and are far less energetic proteolytically.

	Glucose.	Lactose.	Sucrose.	Mannite.	Milk.	Gelatin.	Motility.	Indol.
Vibrio cholerae.....	+	+	+	+	*c*	+	+	+
Vibrio F and *P*.....	+	+	+	+	+	+	+	+
Vibrio Nassac.....	+	+	+	+	*c*	+	+	+
Vibrio H/61.....	—	—	—	—	—	—	+	—
Vibrio H/120.....	—	—	—	—	—	—	+	—
Vibrio Metchnikovi.....	+	—	+	+	*c*	+	+	+

XXVII. B. pyocyaneus. B. prodigiosus.

B. pyocyaneus, the organism of "blue pus," is strongly proteolytic, both in plain and sugar containing glucose broth. Although it is stated to have no action upon glucose, the analyses indicate that it can obtain a certain amount of energy from it, somewhat resembling *B. alcaligenes* in this respect.

B. prodigiosus also an energetic proteolyte changing fully 10% of the protein of sugar-free broth to ammonia in 9 days. It rapidly ferments glucose as well, with the production of a moderate amount of acid. At the end of three days all the glucose is decomposed, so that the protein constituents are rapidly attacked, thus explaining the rapid accumulation of ammonia by the 9th day.

XXVIII. Spore-Forming Group.

With the exception of certain anaerobic spore-forming bacteria, this group is of importance chiefly to the agriculturalist. The unusual proteolytic activity exhibited by many of these organisms, and their ability to withstand unfavorable environmental changes through the resistance

Organism.	Days.	Plain broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	Per cent. NH_3/total N_2.	Glucose broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per cc. broth.	Per cent. NH_3/total N_2.
B. pyocyaneus.....	1	—0.30	—0.30	0.00	23.30	+ 4.40	1.61	—0.60	—0.30	0.00	19.60	+0.70	0.25
	3	—1.60	—0.90	—0.10	27.30	+ 8.40	3.08	+0.70	+0.20	+1.20	18.90	0.00	0.00
	6	—3.60	—1.30	—0.20	44.80	+25.90	9.50	—0.20	+0.60	+0.80	21.70	+ 2.80	1.00
	9	—3.10	—1.60	—0.30	48.30	+29.40	10.80	+2.20	—1.00	+0.60	37.10	+18.20	6.50
B. pyocyaneus, a.....	1	—0.50	—0.30	0.00	17.50	+ 2.10	0.75	—0.30	—0.30	—0.20	15.40	— 0.70	0.22
	3	—2.40	—1.00	—0.30	24.50	+ 9.10	3.25	—0.60	+0.10	+0.20	14.70	— 1.40	0.45
	6	—2.80	—1.70	—0.20	35.70	+20.30	7.25	—0.60	—0.50	+0.20	17.50	+ 1.40	0.44
	9	—3.40	—2.30	—0.20	42.70	+27.30	9.75	—1.70	—2.40	—0.20	38.50	+22.40	6.97
B. prodigiosus.....	1	—0.20	—0.10	0.00	26.60	+ 3.50	1.11	+1.10	+2.50	+2.00	23.80	+ 0.70	0.23
	3	—1.30	—0.60	0.00	36.40	+13.30	4.23	+1.00	+2.10	+1.60	28.70	+ 5.60	1.82
	6	—2.10	—1.30	0.00	56.70	+33.60	10.70	+0.40	+1.30	+1.40	32.90	+ 9.80	3.18
	9	—2.20	—1.60	—0.40	57.40	+34.30	10.90	+0.50	—0.70	—0.10	49.00	+25.90	8.40

Organism.	Days.	Plain broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	Per cent. NH_3/total N_2.	Glucose broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	Per cent. NH_3/total N_2.
B. *anthracis*, I.....	1	0.00	+0.10	+0.10	16.10	+ 0.70	0.25	+1.20	+1.80	+2.00	16.80	+0.70	0.22
	3	—0.40	—0.10	+0.20	18.20	+ 2.80	1.00	+1.20	+1.90	+2.00	16.80	+0.70	0.22
	6	—0.20	+0.10	+0.40	21.00	+ 5.60	2.00	+1.10	+2.10	+1.80	16.80	+0.70	0.22
	9	—0.10	+0.20	+0.40	23.10	+ 7.70	2.75	+2.90	+3.50	+2.70	16.80	+0.70	0.22
B. anthracis, II	1	0.00	+0.10	+0.10	16.10	+ 0.70	0.25	+1.10	+1.50	+1.40	16.80	+0.70	0.22
	3	—0.30	+0.20	+0.20	17.50	+ 2.10	0.75	+1.30	+2.00	+1.80	16.80	+0.70	0.22
	6	0.00	+0.70	+0.60	20.30	+ 4.90	1.75	+1.30	+2.10	+1.80	16.80	+0.70	0.22
	9	0.00	+0.50	+0.40	21.70	+ 6.30	2.25	+2.60	+2.60	+1.90	18.20	+2.10	0.65
B. anthracis, III.....	1	0.00	—0.10	—0.10	16.10	+ 0.70	0.25	+1.20	+1.70	+1.60	16.80	+0.70	0.22
	3	+0.20	—0.10	+0.20	16.80	+ 1.40	0.50	+1.10	+2.10	+1.50	16.80	+0.70	0.22
	6	+0.10	+0.60	+0.50	19.60	+ 4.20	1.50	+1.20	+2.20	+1.70	16.80	+0.70	0.22
	9	—0.30	+0.80	+0.40	21.00	+ 5.60	2.00	+1.90	+1.40	+1.20	18.20	+2.10	0.65
B. mesentericus.....	1	—0.30	—0.10	0.00	16.80	+ 1.40	0.50	+1.50	+2.40	+2.00	16.80	+0.70	0.22
	3	—0.50	0.00	+0.40	27.30	+11.90	4.25	+1.60	+2.50	+2.40	18.20	+2.10	0.65
	6	—0.90	—0.30	+0.40	35.70	+20.30	7.23	+0.80	+2.70	+2.10	18.90	+2.80	0.87
	9	—0.70	—0.40	+0.40	38.50	+23.40	8.26	+1.50	+1.00	+1.70	18.90	+2.80	0.87
B. alvei	1	—0.20	0.00	—0.10	16.10	+ 0.70	0.25	+0.80	+1.80	+1.10	16.10	0.00	0.00
	3	—0.20	0.00	0.00	16.10	+ 0.70	0.25	+1.30	+2.20	+2.00	16.10	0.00	0.00
	6	—0.20	—0.10	+1.00	16.80	+ 1.40	0.50	+0.80	+1.80	+1.50	16.10	0.00	0.00
	9	—0.30	—0.20	—0.10	16.80	+ 1.40	0.50	+1.40	+1.80	+1.70	16.10	0.00	0.00

of their spores, explains their importance in the economy of nature where the rapid degradation of protein to simple compounds, which can be readily transformed into plant food, is a most essential phase in the cycle of the elements. These organisms play a very important part in the nitrogen cycle, transforming the complex nitrogenous compounds of dead organisms rapidly to ammonia, which, in turn, is oxidized successively to nitrites and finally to nitrates by other types of bacteria.

Certain aerobic spore-forming organisms, notably *B. anthracis*, are occasionally parasitic for man and certain animals, notably cattle and sheep. The inability of *B. anthracis* to sporulate *in vivo* is, however, an indication of its saprophytic origin.[1] *B. alvei* is an organism which is stated to be the causative agent in the disease known as "foul brood of bees."

B. mesentericus is widely distributed in nature, and in the intestinal tracts of man and certain animals. It is more actively proteolytic than either *B. anthracis* or *B. alvei*.

	Glucose.	Lactose.	Sucrose.	Mannite.	Milk.	Gelatin.	Indol.	Motility.
B. mesentericus.....	+	—	—	—	*c/p*	+	+	+
B. alvei.....	+	+	+	+	+	—	..	+
B. anthracis.....	+	—	—	—	*c/p*	+	..	—

XXIX. Coccus Group.

The Coccaceae are important pathogenic organisms, many of them being pathogenic for man and the higher animals. They cause a variety of pathological lesions ranging from rapidly fatal septicemia to localized, insignificant abscesses. *Mic. melitensis* causes a slow, intermittent fever variously known as Malta fever, Mediterranean fever and *Febris Undulans*. It is found in the urin and milk of milch goats, through which it is frequently transmitted to man in regions where it is endemic.

Mic. tetragenus is found in unclean mouths, and frequently as a secondary invader in pulmonary tuberculosis. A noteworthy feature of the Coccaceae studied in this series is the formation of acid in sugar-free broth. Whether this is due to their action upon nucleins, upon the "carbohydrate radical" of protein or upon the salts of organic acids which are present in the media is not definitly known. This reaction has been met with before and discussed in some detail by one of us (A. I. K.).[2]

Chemically the organisms show definit and distinctive variations in their action upon protein and carbohydrate, which are brought out in the analytical tables. Culturally their reactions are as follows:

[1] Theobald Smith, *American Medicine*, VIII, Oct. 22, 1904.

[2] Kendall and Farmer, *J. Biol. Chem.*, **12**, 216, 221 (1912).

Organism.	Days.	Plain broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.	Glucose broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.
Streptococcus pyogenes...	1	+0.30	0.00	+0.10	16.80	+ 0.70	0.24	+3.00	+3.40	+3.00	16.10	0.00	0.00
	3	+0.30	+0.70	+0.40	16.80	+ 0.70	0.24	+4.30	+4.60	+4.30	16.10	0.00	0.00
	6	+0.30	+0.50	+0.50	16.80	+ 0.70	0.24	+4.60	+5.20	+4.70	16.10	0.00	0.00
	9	+0.10	+0.70	+0.40	17.50	+ 1.40	0.48	+4.20	+5.00	+4.70	16.80	+0.70	0.24
Streptococcus, No 23....	1	+0.20	0.00	+0.10	16.80	+ 0.70	0.24	+3.00	+3.10	+2.90	16.10	0.00	0.00
	3	+0.40	+0.70	+0.40	16.80	+ 0.70	0.24	+4.10	+4.60	+4.20	16.10	0.00	0.00
	6	+0.30	+0.40	+0.40	16.80	+ 0.70	0.24	+4.70	+5.40	+4.90	16.10	0.00	0.00
	9	+0.20	+0.70	+0.40	17.50	+ 1.40	0.48	+4.20	+4.90	+5.30	16.80	+0.70	0.24
Staphylococcus aureus, I	1	—0.10	0.00	—0.10	16.10	0.00	0.00	+2.60	+2.60	+2.60	16.10	0.00	0.00
	3	+0.10	+0.40	+0.30	16.10	0.00	0.00	+3.40	+3.40	+3.30	16.10	0.00	0.00
	6	—0.50	+0.10	+0.30	24.50	+ 8.40	2.85	+3.50	+3.90	+3.50	16.10	0.00	0.00
	9	—1.40	—0.30	—0.10	36.40	+20.30	6.90	+3.50	+4.20	+3.70	16.80	+0.70	0.24
Staphylococcus aureus, II	1	—0.10	0.00	0.00	16.10	0.00	0.00	+2.60	+2.60	+2.50	16.10	0.00	0.00
	3	+0.30	+0.40	+0.40	16.10	0.00	0.00	+3.20	+3.20	+3.00	16.10	0.00	0.00
	6	—0.30	+0.40	+0.30	19.60	+ 3.50	1.19	+3.30	+3.70	+3.30	16.80	+0.70	0.24
	9	—1.30	—0.20	—0.20	41.30	+25.20	8.60	+2.90	+3.30	+2.80	16.80	+0.70	0.24

Staphylococcus epidermidis albus.....	1	—0.10	0.00	+0.10	16.80	+ 0.70	0.24	+2.40	+2.30	+2.00	16.10	0.00	0.00
	3	+0.10	+0.30	+0.30	16.10	0.00	0.00	+3.60	+3.80	+3.60	16.10	0.00	0.00
	6	+0.30	+0.50	+0.40	17.50	+ 1.40	0.48	+3.70	+3.70	+3.40	16.10	0.00	0.00
	9	—0.10	+0.30	+0.20	18.90	+ 2.80	0.95	+3.50	+4.20	+3.80	16.80	+0.70	0.24
Micrococcus zymogenes.....	1	+0.10	+0.10	+0.20	17.50	+0.70	0.26	+4.00	+3.70	+3.30	16.80	0.00	0.00
	3	+0.40	+0.50	+0.20	17.50	+0.70	0.26	+4.70	+5.50	+4.90	16.80	0.00	0.00
	6	+0.10	+0.60	+0.20	17.50	+0.70	0.26	+4.40	+5.30	+4.50	17.50	+0.70	0.25
	9	+0.10	+0.60	+0.40	18.20	+1.40	0.50	+5.90	+5.70	+4.70	17.50	+0.70	0.25
Micrococcus melitensis..	1	0.00	0.00	0.00	16.10	0.00	0.00	+0.70	+2.00	+1.60	16.10	0.00	0.00
	3	+0.20	+0.40	+0.50	16.10	0.00	0.00	+2.90	+3.20	+3.10	16.10	0.00	0.00
	6	—0.30	+0.10	+0.20	18.90	+2.80	0.96	+3.20	+3.20	+2.90	16.80	+0.70	0.24
	9	—0.50	—0.10	+0.20	22.40	+6.30	2.14	+3.30	+3.50	+3.10	16.80	+0.70	0.24
Micrococcus tetragenus.....	1	—0.20	—0.10	0.00	15.40	—0.70	0.24	+0.40	+1.20	+1.10	16.10	0.00	0.00
	3	+0.30	+0.60	+0.40	16.10	....	0.00	+2.70	+2.80	+2.80	15.40	—0.70	0.24
	6	+0.50	+1.00	+0.80	16.80	+0.70	0.24	+3.10	+3.40	+3.10	16.10	0.00	0.00
	9	+0.60	+1.00	+0.80	18.20	+2.10	0.71	+2.90	+3.20	+3.00	16.80	+0.70	0.24

Organism.	Days.	Plain broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 cc. broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.	Glucose broth. Alizarin.	Neutral red.	Phenolphthalein.	NH_3 mg. per 100 broth.	NH_3 mg. increase per 100 cc. broth.	NH_3/total N_2. Per cent.
B. leprae, "Duval".....	1	0.00	+0.10	+0.10	16.10	+ 0.70	0.25	+1.20	+1.50	+1.30	16.10	0.00	0.00
	3	—0.30	—0.20	—0.20	15.40	0.00	0.00	+1.10	+1.60	+1.30	16.10	0.00	0.00
	6	0.00	0.00	0.00	16.80	+ 1.40	0.50	+0.50	+2.20	+1.70	16.10	0.00	0.00
	9	—0.40	0.00	0.00	21.00	+ 5.60	2.00	+1.30	+2.80	+1.80	18.20	+2.10	0.65
B. leprae, "Hardy".....	1	0.00	—0.10	0.00	15.40	0.00	0.00	+0.10	+0.10	0.00	16.10	0.00	0.00
	3	0.00	0.00	+0.10	15.40	0.00	0.00	+1.30	+1.80	+1.40	16.80	+0.70	0.22
	6	—0.50	—0.40	—0.30	15.40	0.00	0.00	—0.30	—0.40	—0.50	15.40	—0.70	0.22
	9	—0.60	—0.20	—0.50	15.40	0.00	0.00	—0.30	—0.20	—0.40	16.10	0.00	0.00
Nasal secretion, "Karlinski"	1	+0.10	0.00	0.00	16.10	+ 0.70	0.25	+0.10	+0.10	+0.10	16.10	0.00	0.00
	3	—0.30	0.00	—0.20	16.10	+ 0.70	0.25	—0.30	0.00	—0.40	15.40	—0.70	0.22
	6	—0.50	—0.30	—0.20	17.50	+ 2.10	0.75	0.00	—0.10	—0.30	9.80	—6.30	1.95
	9	—0.60	—0.50	—0.30	19.60	+ 4.20	1.50	—0.30	—0.50	—0.60	11.20	—4.90	1.52
Moeller's grass bacillus, II	1	—0.10	—0.10	—0.10	15.40	0.00	0.00	0.00	+0.10	—0.10	16.80	+0.70	0.22
	3	—0.30	—0.20	—0.10	16.10	+ 0.70	0.25	—0.10	—0.20	0.00	13.30	—2.80	0.87
	6	—1.50	—0.60	—0.60	20.30	+ 4.90	1.25	—0.80	—0.50	—0.50	10.50	—5.60	1.74
	9	—1.90	—0.90	—0.80	28.00	+12.60	4.50	—0.70	—0.80	—1.10	10.50	—5.60	1.74
Moeller's grass bacillus, III	1	—0.20	0.00	—0.10	16.10	+ 0.70	0.25	0.00	0.00	0.00	16.10	0.00	0.00
	3	—0.80	—0.20	—0.50	15.40	0.00	0.00	—0.70	—0.20	—0.40	13.30	—2.80	0.87
	6	—0.60	—0.60	—0.70	20.30	+ 4.90	1.25	—1.10	—0.50	—0.60	7.70	—8.40	2.60
	9	—1.50	—0.80	—0.80	29.40	+14.00	5.00	—0.60	—0.60	—1.10	7.70	—8.40	2.60
Moeller's grass bacillus, IV	1	—0.10	—0.30	—0.10	16.10	+ 0.70	0.25	—0.10	+0.10	—0.10	16.10	0.00	0.00
	3	—0.50	—0.10	—0.10	15.40	0.00	0.00	—0.30	—0.20	—0.40	14.00	—2.10	0.65
	6	—1.40	—0.50	—0.80	21.00	+ 5.60	2.00	—0.90	—0.40	—0.50	9.80	—6.30	1.95
	9	—1.70	—0.80	—0.80	28.70	+13.30	4.75	—0.70	—0.60	—1.10	8.40	—7.70	2.40

	Glucose.	Lactose.	Sucrose.	Mannite.	Milk.	Gelatin.	Indol.
Streptococcus pyogenes.....	+	+	+	+	*c*	—	—
Staphylococcus pyogenes.....	+	+	+	+	*c/p*	+	—
Mic. tetragenus.....	+	+	+	+	*c*	—	—
Mic. melitensis.....	+	+	+	+	+	—	—
Mic. zymogenes.....	+	+	+	+	*c/p*	+	—

XXX. The Acid Fast Group.

This group is a very extensive one, possessing, in common, a waxy envelope which comprises from 15 to 30% of the total dried weight of the organisms, and which confers on them their remarkable staining properties. Dynamically there is the widest range in their activities from the exquisitely pathogenic *B. tuberculosis* and *B. leprae* to the very ubiquitous grass bacilli.

The members of the group considered here comprise three strains of Müller's grass bacillus, *B. leprae* "Hardy" from the Hygienic Laboratory in Washington, *B. leprae* "Duval" from New Orleans and the organism known as the "Nasal Secretion" bacillus of Karlinski. The latter clearly belongs to the grass bacillus type.

A prominent chemical characteristic of the grass bacilli is the "negative ammonia phase" in glucose broth. This is also exhibited by the "Nasal Secretion" culture.

Leprosy culture "Hardy" produced practically no measurable change in either plain or glucose broth, although it grew with moderate luxuriance. The group as a whole is culturally inactive.

[CONTRIBUTIONS FROM THE CHEMICAL LABORATORIES OF COLUMBIA UNIVERSITY, NO. 216.]

THE WEIGHT OF A FALLING DROP AND THE LAWS OF TATE. XI. THE DROP WEIGHT AND SURFACE TENSION OF BLOOD SERUM.[1]

BY J. LIVINGSTON R. MORGAN AND HAROLD E. WOODWARD.

Received July 17, 1913.

Object of the Investigation.

In a series of papers from this laboratory[2] it has been shown by one of us, with co-workers, that the weight of a drop of liquid falling from a properly constructed tip, as determined by aid of a special apparatus (which

[1] We are deeply indebted in this work to Drs. Bailey, Hopkins and Smith of St. Luke's Hospital, to Drs. Butterfield and Bronfenbrenner of the Rockefeller Institute, and to Dr. Warren of Roosevelt Hospital, and take this occasion to express our thanks to them.

[2] THIS JOURNAL, **30**, 360–76, 1055–68; **33**, 349–62, 643–57, 657–72, 672–84, 1041–60, 1060–71, 1275–90, and 1713–27.