A most fundamental principle of bacterial metabolism may be expressed concisely by stating that "Fermentation takes precedence over putrefaction;"1 that is to say, bacteria in general which can utilize both carbohydrate and protein, act upon the former in preference to the latter when both are present in the same medium.

In view of the confusion attending the use of the terms putrefaction and fermentation, they must be sharply defined. By fermentation is meant "the action of microorganisms upon carbohydrate," and by putrefaction is meant "the action of microorganisms upon nitrogenous substances."2 Bacteria in common with all known living things need nitrogen to build up their bodies; it is self-evident, therefore, that even when carbohydrate is being fermented, enough protein must be broken down to satisfy their nitrogen requirements. Bacterial activity, therefore, must be sharply differentiated into two distinct processes, the structural and the vegetative, both functions being essential for their metabolism.

Nitrogen is indispensable for the structural process, hence bacteria must have nitrogen in their dietary. With the vast majority of bacteria, however, the vegetative process may be satisfied either by utilizable carbohydrate or by protein. Whenever bacteria can utilize both carbohydrate and protein for their vegetative activity (for fuel) and both are present in the medium in which these

2 For a more complete discussion of these terms, see Kendall: Journ. of Med. Res., pp. 140-144, 1911.
organisms are growing, the carbohydrate is invariably selected in preference to the protein. Even when protein is being utilized for vegetative purposes, the bacteria actually eliminate nitrogen from the protein molecule and apparently utilize only the carbon, hydrogen and oxygen for their energy metabolism (fuel). This amounts practically to the use of carbohydrate in the last analysis for fuel purposes.

In the past, but little effort has been made to study bacterial metabolism quantitatively, at least from the comparative standpoint, yet it is largely from the comparative study of different types of bacterial metabolism that the fundamental principles can be elucidated. This lack of diligence cannot be explained wholly by the inadequateness of the older methods; it is rather attributable to rigid adherence to the narrow, botanical idea of morphology and differentiation of bacteria instead of the broader, dynamical consideration of bacterial activity. The few painstaking studies which have been made along these lines have failed for the most part because of the neglect of the carbohydrate factor in the media in which such experiments have been carried out. For these reasons, it is impossible to quote from the literature any studies which are carried out along lines similar to those presented below.

The results upon which this summary is based have been derived from the comparative study of a considerable number of bacterial types using methods of far greater accuracy than those previously available. The types of bacteria represented in this series cover those commonly met with in bacteriology, and a sufficiently large number of strains of each type have been examined to give definite assurance of the physiological and chemical limits of each species. The organisms selected for this work have been drawn largely from the normal and pathological flora of the gastro-intestinal tract where dietary alternations, comparable with those to which these bacteria are subjected culturally, are commonly met with.

The methods employed have been described critically in previous communications, and will not be referred to here other than to state that the limits of error are much less than the thickness of the lines shown in the various curves at the end of the paper.

Similarly, the analytical results have been presented in previous

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articles. It is the purpose of this communication to plot, analyze and synthesize these results. The appended curves, accurately constructed from these analytical figures, show graphically the effects of carbohydrate upon bacterial metabolism. These curves furthermore illustrate in a striking manner the diversity of types of bacterial metabolism in media of the same composition; yet in spite of these diversities the sparing action of carbohydrate for protein is apparent, except in the strictly "carnivorous" organisms, *B. alcaligenes* and H-61.

The frankly pathogenic organisms associated with toxemia in the human body, as typhoid and dysentery (both Shiga and Flexner), break down but little protein, as is shown by the small amount of ammonia liberated in the sugar-free medium, when they are using protein for fuel as well as for structural purposes. The amount of ammonia liberated by the less frankly pathogenic organisms increases progressively as the more saprophytic types, *e.g.*, *B. proteus*, are approached. At first sight, cholera might seem to be an exception to this generalization. It must be remembered, however, that cholera may be a rapidly fatal disease, the entire course from infection to death taking place within twelve hours. This contrasts strikingly with diseases such as typhoid, where the incubation period alone is about fourteen days on the average. The proteolytic activity of cholera may be effectively checked by the presence of dextrose.

It will be seen from the curves that the proteolytic activity, but not the structural activity of bacteria (except the strictly carnivorous types), can be arrested by the presence of utilizable carbohydrate. The products of proteolytic activity, which are only formed when bacteria are utilizing protein for fuel, are alkaline, nitrogenous substances; the products of fermentation, on the contrary, which are formed when bacteria are utilizing carbohydrate for fuel, are non-nitrogenous, acid products. It must be remembered that all known true toxins are nitrogenous, while acids produced by fermentation are at best but irritants and are for the most part non-nitrogenous. It would appear, therefore, that the production of toxic substances of bacterial origin must be the result of proteolytic (putrefactive) activity rather than of fermentative activity.

The importance of the sparing action of carbohydrate for protein in the light of toxin production must be apparent.

We believe that the principle elucidated above is not limited to bacteria alone, but that it is in reality a general and fundamental principle of cellular metabolism.

EXPLANATION OF CHARTS.

The solid, heavy line represents the production of ammonia in milligrams of nitrogen per 100 cc. of culture medium in sugar-free broth.

The solid, light line represents the production of ammonia in milligrams of nitrogen per 100 cc. of culture medium in dextrose broth.

The broken heavy line represents reaction in terms of cubic centimeters of normal acid or alkali per 100 cc. of sugar-free culture medium.

The broken light line represents reaction in terms of cubic centimeters of normal acid or alkali per 100 cc. of dextrose broth.

The composite curve illustrates graphically the relative amounts of ammonia produced by various types of pathogenic and saprophytic bacteria; for convenience the different types are drawn in the same figure to bring out forcibly the difference in ammonia production in sugar-free broth between these types. It will be seen that the production of ammonia in dextrose broth is essentially the same for all these organisms, excepting those which can utilize no sugar. This ammonia production in sugar-containing broth is a measure of the nitrogen needs of bacteria for structural purposes as contrasted with the fuel needs.
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Composite Curve

MgN per 100cc

Days I II III IV V VI VII VIII

Proteus

Coli

Paratyphoid

Typhoid-Shiga, Flexner

Structural Nitrogen

Composite Curve