BACTERIAL METABOLISM

THE INFLUENCE OF PHOSPHATE BUFFER IN CARBOHYDRATE-FREE AND IN GLUCOSE-CONTAINING MEDIA

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Many attempts have been made to follow the metabolic changes brought about by bacteria in mixed and in pure culture in various kinds of media, but the complexity of the many deep-seated changes which take place in the organic substrates is such that it may be said but little progress has been made in this field of biochemistry.

Much attention has been directed in more recent years to a study of the immediate fate of complex nitrogenous substances, as revealed by ammonia production and by changes in amino acid content of the medium, and of the ability of microörganisms to destroy various carbohydrates, higher alcohols, etc., with the resultant production of acids and other products characteristic of such decomposition.

A subject of particular interest to investigators has been the influence of the presence of utilizable carbohydrates on the course of nitrogen metabolism, with not a little difference of opinion as to the real significance and explanation of altered metabolism brought about by such agents.

As early as 1886 Hirschler observed that the production of indol, phenol and cresols is inhibited by the presence of available carbohydrate.

Péré (1892) showed that, although sugar has a restraining effect on indol formation, the indol-producing function is not permanently lost. Inoculation from a fifteenth generation glucose broth culture into plain peptone resulted in the restoration

of the original proteolytic property of the organism. Péré claimed further (1893) that the relative amount of acid formed by the colon bacillus decreases with greatly increased amounts of peptone in the medium.

Peckham (1897) concluded that when members of the colon group are cultured under conditions favorable to the development of both proteolysis and fermentation, the latter invariably takes precedence, and that there is no evidence of proteolysis until fermentation has ceased.

De Graaf (1909) noted that glucose exerts an inhibitory effect on indol production. Rougentzoff (1913) reported that glucose, fructose, lactose and mannitol inhibit indol formation by *Bact. coli*. Indol was formed, however, in media containing sucrose, maltose and dulcitol.

Distaso (1913) was of the belief that in a test medium containing utilizable carbohydrate and tryptophan as the source of indol, the more readily available substances are used first by *Bact. coli*, and that consequently indol is not formed in ordinary sugar media. The above observations have been on the whole confirmed by numerous investigators.

Kendall, Day and Walker (1913) conducted a series of studies on the relative constancy of ammonia production by *Bact coli* and *Bact. typhosum*. They observed very little or no increase in ammonia in peptone broth containing glucose, whereas ammonia production in carbohydrate-free broth could be readily demonstrated. These and similar observations with other organisms led Kendall to maintain that fermentation takes precedence over putrefaction, and that glucose exerts a sparing action on protein when both are present. In their later work, Kendall and his associates extended their quantitative studies to amino nitrogen, total nitrogen and non-protein nitrogen, along with the ammonia determinations.

Liborius (1886) found that the presence of sugar did not inhibit gelatin liquefaction in cultures of *B. subtilis*, *Ps. fluorescens* and *Ps. pyocyanea*.

Sears (1915) observed that generally the concentration of free ammonia and amino acids was lower in glucose than in plain broth.

B. subtilis and *Vibrio metchnikovi* proved to be exceptions. *Cl. Welchii*, on the other hand, produced more ammonia and amino acids in the presence of glucose than in its absence.

Glenn (1911) concluded that indol production by *Bact. coli* and *Proteus vulgaris* is inhibited in sugar-containing media by the acid formed, and not by the carbohydrate *per se*.

Waksman (1920) held that a definite amount of ammonia may always be produced out of complex nitrogenous compounds, whether available carbohydrate is present or not, but that the ammonia may be utilized in the presence of the sugar, while in a sugar-free medium proteolytic organisms utilize the "protein" substances as a source of carbon as well as of nitrogen, leaving large quantities of ammonia in the medium.

Henderson and Webster (1907) found that the presence of phosphate greatly favors the growth of *Bact. acidi-lactici* (coli). They explained this function by the ability of phosphates to neutralize the acid, with the reservation that their observations did not conclusively establish this claim.

Fischer (1915) held that acidity plays no rôle in the inhibition of indol formation in sugar-containing media. Kligler (1915) maintained that while acid does inhibit liquefying enzymes, it is usually the sugar that is directly responsible for the absence of liquefaction. Jones (1916) attributed the failure of the enzymes of *Proteus vulgaris* to develop in sugar-containing medium to a paralyzing action of hydrogen ions on the endo-enzyme.

Kligler (1916) found that with moderate amounts of glucose present, ammonia production increased with increased concentration of peptone. He suggested that the greater ammonia production may have been due to the favorable buffer effect of increasing amounts of peptone.

Berman and Rettger (1918) observed that the presence of sufficient buffer (phosphate) in glucose-containing medium encourages continued normal nitrogen metabolism of *Proteus vulgaris*. The phosphate also tended to favor normal nitrogen metabolism of *Bact. coli* in the presence of relatively small amounts of glucose.

B. subtilis, though a glucose-utilizing organism, was able to

maintain its proteolytic activity in the presence of this sugar, without added buffer. This continued metabolic function was explained by Berman and Rettger by the assumption that the nitrogenous substances are attacked by this organism relatively easily, and that the organism is thus able to produce its proteolytic enzyme and consequently its own buffer to offset the free H ions formed from the glucose. They concluded that moderate amounts of utilizable carbohydrate do not affect the nitrogen metabolism of bacteria, provided favorable experimental conditions are maintained. The more luxuriant growth of organisms in glucose phosphate medium was explained on the ground that the nitrogenous constituents of the medium are utilized for structural purposes for which the glucose furnishes much of the energy supply, whereas in the plain broth the nitrogenous substrate furnishes both the structural and functional food requirements.

Bronfenbrenner and Schlesinger (1918) made a study of the metabolism of *Bact. coli* in peptone-phosphate-lactose water, and concluded that careful consideration should be given to the relation of buffer salts to carbohydrates in the conducting of fermentation experiments.

De Bord (1923) carried on metabolism studies in buffered (phosphate) medium. His results indicated that the production of amino acids was always greater in glucose than in plain broth, but that ammonia production was much greater in the sugar-free than in the glucose broth. He believed, as did Raistrick and Clark (1921), that free ammonia is not a reliable index of bacterial proteolysis.

EXPERIMENTAL

The main purposes of this investigation were to determine the influence of balanced phosphate on the course of nitrogen metabolism in glucose-containing and in carbohydrate-free peptone media, and to make a comparative study of the metabolic activities of certain selected organisms representing widely different types and groups.

The work involved a systematic quantitative determination of

total, protein, non-protein, ammonia, amino, and polypeptid nitrogen, and biuret and glucose. Also H ion concentration and turbidity.

The following 22 organisms were employed: *B. anthracis*, *B. subtilis* (2 strains), *B. cereus*, *B. megatherium*, *Bact. coli* (2 strains), *Bact. typhosum* (2 strains), *Bact. paratyphosum* A (2 strains), *Bact. paratypohsum* B (2 strains), *Bact. pullorum* (2 strains), *Proteus vulgaris*, *Bact. bronchisepticum*, *Ps. pyocyanea*, *E. prodigiosus*, *Staph. aureus*, *Cl. sporogenes*, *Cl. welchii*.

A solution of 1 per cent Witte peptone was used as the basic medium. This was employed as

(1) Plain peptone without glucose
(2) Plain peptone plus 1 per cent glucose
(3) Plain peptone plus phosphate buffer, without glucose
(4) Plain peptone plus phosphate buffer and 1 per cent glucose

In a few instances ammonium sulphate was also used to determine the fate of the added ammonia in the different media.

The buffer solution was made by dissolving 12 grams of K_2HPO_4 and 5 grams of NaH_2PO_4 in 200 cc. of distilled water. This mixture of the primary and secondary phosphates yielded a solution having a H ion concentration of pH 6.8. Sufficient amount of this was added, sterile, to the sterile media to give a concentration of 1 per cent of the dry salt mixture, or approximately M/7. The pH of the final media was 6.8. All of the media were tubed in 8 by 1 inch test tubes, 40 cc. to the tube. Inoculation was made from twenty-four-hour slant agar cultures, except in the case of the two anaerobes where inoculations were made directly from broth cultures. Efforts were made to make the amounts of inoculum small and as nearly uniform as possible.

Incubation was conducted at the optimum temperatures of the given organisms.

The metabolic changes were determined as follows:

Total nitrogen, by the Gunning modification of the Kjeldahl procedure

Non-protein nitrogen, by the Folin and Wu method for the determination of non-protein nitrogen in the blood

Protein nitrogen, by subtracting the non-protein from the total nitrogen figure

Ammonia nitrogen, by Van Slyke and Cullen's modification of the Folin air current method

Amino nitrogen, by the micro-method of Van Slyke (1913–1914, 1915) and by the Brown modification (1923) of the technique of Henrique and Sörensen

Quantitative Biuret, by the method of Vernon (1904)

Glucose, by the method of Benedict (1911), involving the use of potassium thiocyanate and copper sulphate

H Ion Concentration, by the colorimetric method of Clark and Lubs (1917)

Turbidity (approximate), by means of the McFarland nephelometer system (1907) and a comparator block

In a preliminary study of the influence of glucose on the metabolism of *B. subtilis*, *B. cereus*, *Proteus vulgaris* and *Bact. coli*, without added buffer, the following observations were made.

B. subtilis, a glucose-utilizing organism, is able to elaborate a proteolytic enzyme in the presence of glucose and to effect nitrogenous changes which are comparable or may even exceed those produced in plain peptone. The amino nitrogen figures for the 0.5 and 1.0 per cent peptone containing 1 per cent glucose were higher than for the plain peptone tubes, while the ammonia figures were variable, but ran fairly parallel in both media.

On the other hand, *B. cereus*, a closely related member of the subtilis group, presented a different type of nitrogen metabolism. The glucose was rapidly fermented and a H ion concentration inimical to proteolytic activity was soon reached. In plain peptone the nitrogenous changes closely resembled those of *B. subtilis*, that is, *B. cereus* brought about considerable increase in amino and ammonia nitrogen; in the glucose peptone solution, however, very little increase could be demonstrated.

Proteus vulgaris also produced a distinct acidity in glucose peptone which was sufficient markedly to inhibit proteolytic activity. There was an actual apparent loss of amino and ammonia nitrogen in the glucose medium, which may perhaps be explained by the assumption that the simple amino acids and ammonia were used

for structural purposes, with no compensatory production of these substances.

Glucose exerted a restraining action on *Bact. coli* quite similar to that of *Proteus vulgaris*. There was abundant evidence that this organism also utilized ammonia for growth, in the presence of glucose, and in some instances reduced it to zero. On the other hand, *Bact. coli* caused a distinct increase in amino and ammonia nitrogen in the glucose-free medium.

In a second preliminary experiment simple comparative metabolic studies were made on *B. anthracis*, *Bact. coli* and *Bact. paratyphosum* A and B, in plain and in glucose peptone.

The amino, non-protein and ammonia nitrogen figures indicate that the metabolic activities of *B. anthracis* in plain and in glucose peptone are quite similar to those of *B. cereus*. The rapid change in H-ion concentration in the presence of glucose from pH 6.9 to pH 4.8 within twenty-four hours readily explains why metabolic activity of *B. anthracis* is so profoundly influenced (retarded) by the glucose.

The nitrogenous changes produced by *Bact. coli* in plain peptone were slight as compared with those of the subtilis group. There was a noticeable increase in amino, ammonia and non-protein nitrogen between the tenth and the thirtieth days of incubation, whereas a decrease in these substances was observed in the glucose-containing tubes. This again is to be expected from the rapid increase in H-ion concentration to pH 4.6 in twenty-four hours.

The nitrogenous metabolism of *Bact. paratyphosum* A and B was quite similar in many respects to that of *Bact. coli*. Amino nitrogen decreased in both the plain and glucose peptone during the first ten days. After this there appeared to be an increase. The small amount of ammonia present at the beginning in the glucose-containing medium disappeared completely, while in the plain medium some increase took place.

RESULTS OBTAINED IN EXPERIMENTS INVOLVING THE USE OF BUFFER

The phosphate buffer exerted a marked influence on the nitrogen metabolism of many of the organisms studied. Its effect on

closely related types or species varied widely in a number of instances, as, for example, with certain members of the subtilis group.

Bacillus subtilis, Bacillus cereus and Bacillus megatherium

B. subtilis and *B. cereus* produced free ammonia more rapidly in the buffered sugar-free medium than in the plain (figs. 1, 2 and 3), whereas little ammonia was formed by *B. megatherium* in the buffered plain peptone water, but an appreciable amount in the unbuffered (fig. 4).

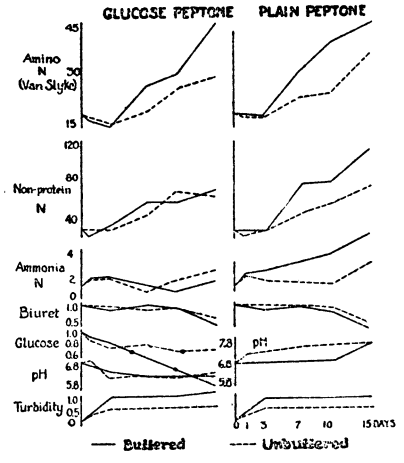

FIG. 1

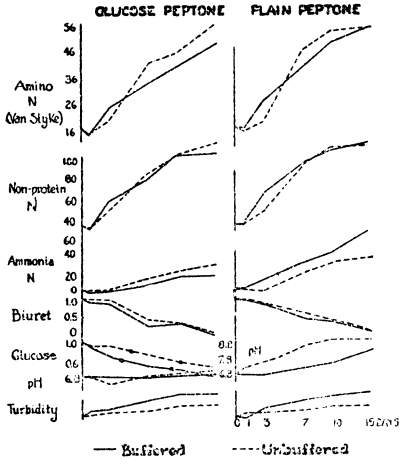

FIG. 2

FIG. 1. BACILLUS SUBTILIS (SHELTEN)
FIG. 2. BACILLUS SUBTILIS (TAP WATER)

Similarly, the amino nitrogen figures for both *B. subtilis* and *B. cereus* were generally higher for the buffered tubes than the unbuffered, while those of *B. megatherium* were appreciably lower.

B. subtilis revealed essentially the same kind and degree of changes in glucose as in plain peptone, and in the buffered glucose as in the plain glucose medium. *B. cereus* and *B. megatherium*, on the other hand, were influenced very little by the phosphate in the glucose medium, and there was comparatively little proteolytic action in both the buffered and the unbuffered glucose

peptone water, due to the rapid increase of H-ion concentration. It may be assumed that the unfavorable influence of the phosphate on the metabolism of *B. megatherium* in the sugar-free medium is due to the slower action of the proteolytic enzyme at the lower H-ion concentration, pH 6.8.

The biuret and non-protein nitrogen figures are in agreement with the changes indicated by the free ammonia and amino nitrogen determinations. Glucose consumption was in all three instances greater in the buffered than in the unbuffered solutions.

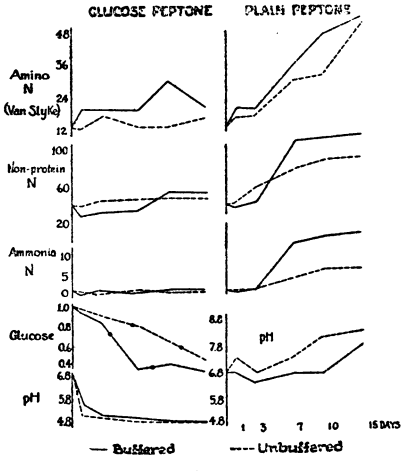

FIG. 3

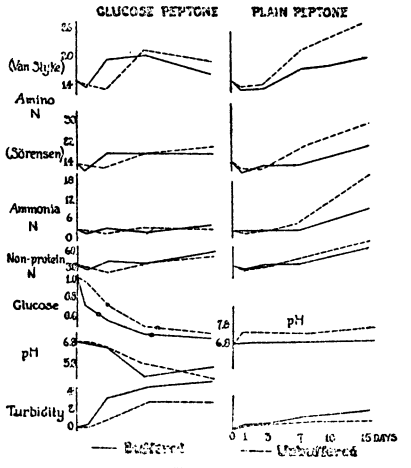

FIG. 4

FIG. 3. BACILLUS CEREUS (HAY INFUSION)
FIG. 4. BACILLUS MEGATHERIUM (N. Y. U.)

The growth curves, when given, correlate with those showing glucose consumption; in other words, the balanced phosphate favored bacterial development.

Erythrobacillus prodigiosus

Erythrobacillus prodigiosus showed a type of nitrogen metabolism in unbuffered plain and glucose peptone very similar to that indicated for *B. cereus* (see fig. 5). In the former medium a rapid increase in amino, non-protein, and ammonia nitrogen occurred, while in the unbuffered glucose peptone tubes virtually

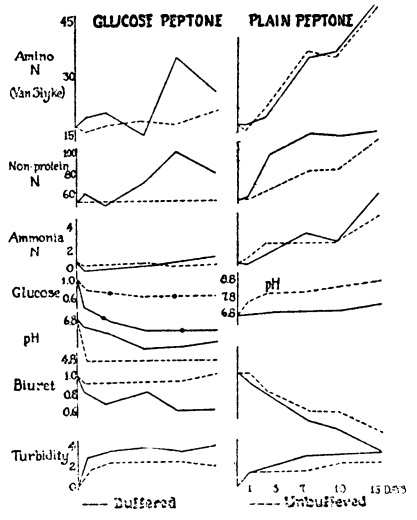

FIG. 5

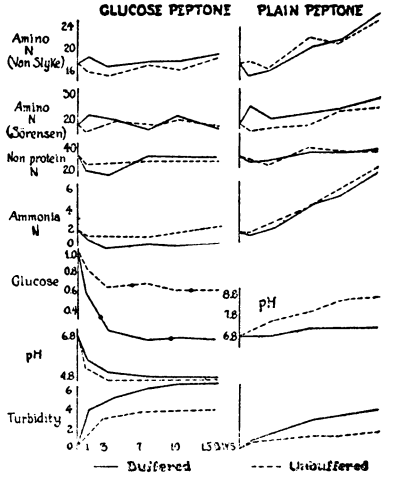

FIG. 6

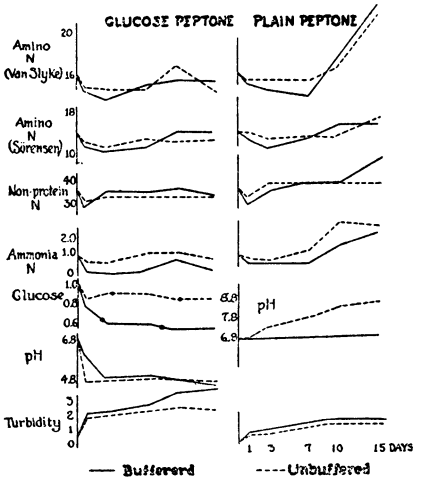

FIG. 7

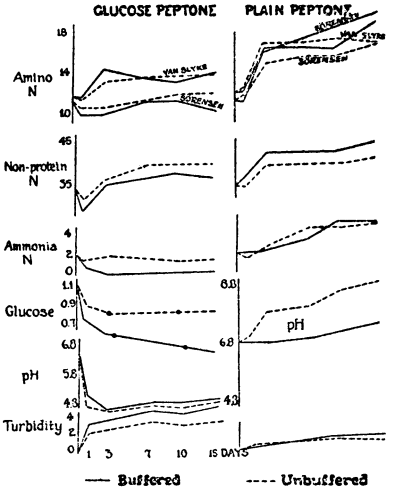

FIG. 8

FIG. 5. ERYTHROBACILLUS PRODIGIOSUS
FIG. 6. BACTERIUM COLI
FIG. 7. BACTERIUM TYPHOSUM (R)
FIG. 8. BACTERIUM PARATYPHOSUM A (Kr. 207)

no changes could be detected in these nitrogen fractions. The rapid drop in pH, from 6.8 to 4.8, within twenty-four hours explains the lack of proteolytic activity in the sugar-containing cultures. Added phosphate, however, prevented this rapid change in H-ion concentration and elicited considerable proteolytic action in the presence of glucose.

A distinct increase in amino and non-protein nitrogen was observed in the buffered glucose-containing cultures, with a simul-

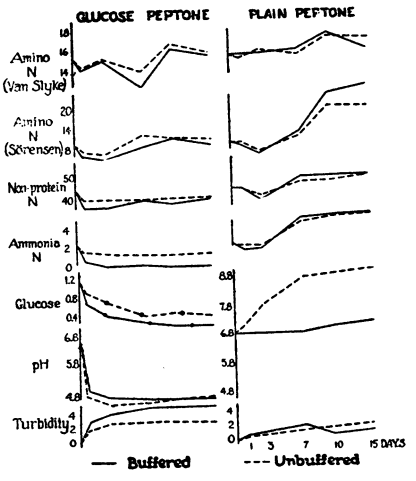

FIG. 9

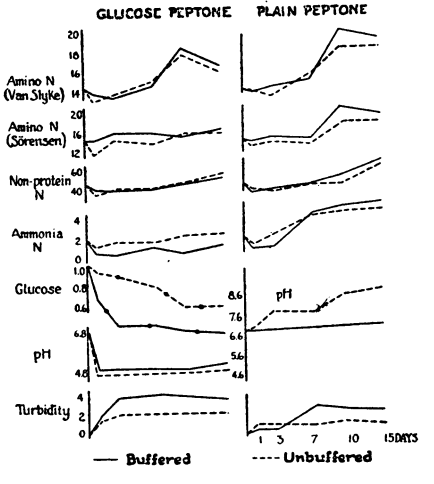

FIG. 10

FIG. 9. BACTERIUM PARATYPHOSUM B (J-12)
FIG. 10. BACTERIUM PULLORUM (Y-24)

taneous decrease in the biuret figures. Glucose utilization was approximately three times as great in the buffered tubes as in the unbuffered, and the turbidity values were noticeably higher.

The addition of phosphate to the plain medium resulted in a slight acceleration of the nitrogen metabolism of this organism. The more luxuriant growth in the buffered tubes seems to be directly associated with the favorable regulatory influence of the phosphate on OH ion concentration.

Bacterium coli, Bacterium typhosum, Bacterium paratyphosum A, Bacterium paratyphosum B, and Bacterium pullorum

These five organisms are grouped together here because of their general relationships and the close similarity, on the whole, of their metabolic activities (see figs. 6, 7, 8, 9 and 10).

All showed a decrease, though in some instances very slight, in the earlier stages, in amino, non-protein, and ammonia nitrogen. *Bact. typhosum* and *Bact. paratyphosum* A and B utilized more nitrogen in the buffered glucose-containing medium than in the glucose-free. After the early, and in a few instances rather prolonged, decrease in amino nitrogen, there was a very apparent increase which, in the glucose-free tubes, was quite pronounced for the different organisms.

Ammonia production, as judged by the observed figures, was quite marked after the early drop in the curves, especially after the first one or two days. The increase in accumulated ammonia in the sugar-free tubes was about the same in those which contained the phosphate as in those which did not. Ammonia production in the glucose-free peptone by *Bact. coli* was apparent from almost the beginning, and continued to the end of the experiment, with a total ammonia yield far beyond that of all of the other four organisms.

Glucose utilization was in every instance much greater in the phosphate-containing than in the unbuffered tubes. The buffer also reacted very favorably on the growth of all of the five organisms in the glucose-containing tubes.

The amount of phosphate used in the sugar-containing peptone was not sufficient to prevent marked increase in H-ion concentration, though a slight buffering action was apparent. On the other hand, the buffer maintained an almost constant pH in the glucose-free tubes throughout each of the experiments.

Proteus vulgaris

Proteus vulgaris displayed metabolic changes in buffered glucose peptone which in the main were quite similar to those in the plain peptone (see fig. 11). Apparently the amount of phos-

phate added proved to be sufficient to maintain the H-ion concentration within suitable growth limits and to favor continued normal nitrogen metabolism. Free ammonia production in the buffered glucose medium approximated that which occurred in the plain peptone, but in the unbuffered glucose broth little change took place. The initial decrease in free ammonia may be ascribed to utilization of that nitrogen fraction by the bacteria for structural purposes.

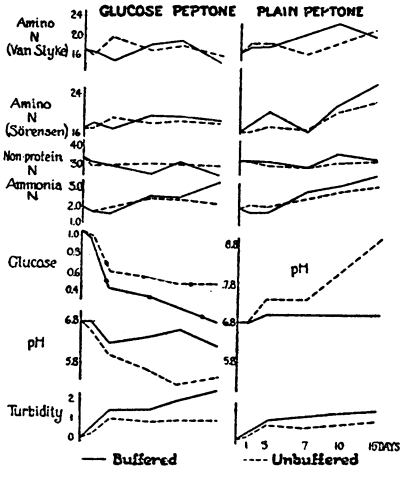

FIG. 11

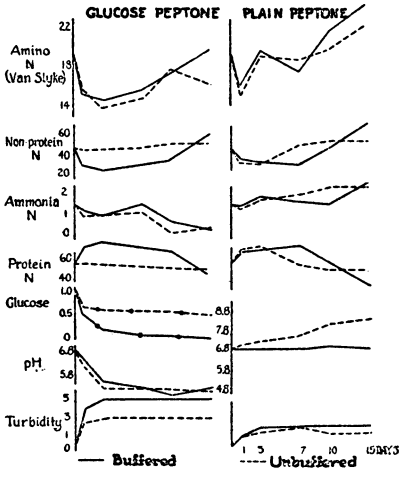

FIG. 12

FIG. 11. PROTEUS VULGARIS (N. Y. U.)
FIG. 12. STAPHYLOCOCCUS AUREUS

Somewhat higher amino, non-protein and ammonia nitrogen values were found in the buffered plain broth cultures than in the unbuffered. Both growth and glucose utilization were greater in the cultures which contained added phosphate than in those which did not. The acceleration of metabolic activities apparently bears a direct relation to the favorable pH maintained by the balanced phosphate mixture.

Staphylococcus aureus

The metabolism of *Staphylococcus aureus* was similar in many ways to that of the colon-typhoid group (fig. 12). During the

first seven days of growth utilization of amino nitrogen in all of the tubes was very pronounced, amounting to approximately 20 per cent of the total amino nitrogen content of the control medium. The buffer exerted little influence in either the plain or the glucose medium. Free ammonia decreased noticeably and practically disappeared from both glucose cultures by the fifteenth day.

The turbidity of the buffered glucose-containing culture was about twice as great as that of the corresponding unbuffered tube and the difference in the amount of sugar consumption was approximately the same.

The plain peptone cultures of *Staph. aureus* gave some indication of proteolytic activity after the first seven days of growth. Amino and non-protein nitrogen increased noticeably after this period in both the buffered and unbuffered plain medium, but somewhat more rapidly in the tubes containing added phosphate than in the others.

Although the buffer exerted only a slight regulatory effect on H-ion concentration in the sugar medium, it maintained a uniform pH in the plain peptone.

Bacterium bronchisepticum

Although the nitrogen metabolism of *Bacterium bronchisepticum* was slight, it proved to be unique in respect to changes in the amino nitrogen fraction (fig. 13). The Van Slyke figures show a progressive decrease in amino nitrogen with a slight concomitant increase in free ammonia. The lower non-protein nitrogen values of the buffered cultures are to be expected, in view of the much more luxuriant growth obtained in the buffered medium, both the glucose and the plain. The increased growth seems to be directly associated with the maintenance of a favorable pH by the buffer during the entire fifteen-day period of observation, the phosphate holding the H-ion concentration at or near pH 6.8 as compared with an increase in pH to 8.0 or slightly above in the unbuffered medium.

The presence of glucose did not affect the metabolism of this organism. Glucose determinations were made here, but it is

quite probable that there was very little glucose utilization at best. The increased turbidity in the buffered peptone tubes may be ascribed, in a small measure, to the phosphate as such, aside from its buffer action.

Pseudomonas pyocyanea

Ps. pyocyanea exhibited a unique type of nitrogen metabolism in the presence of added phosphate (fig. 14). The changes brought about by this organism in amino and non-protein nitrogen in the

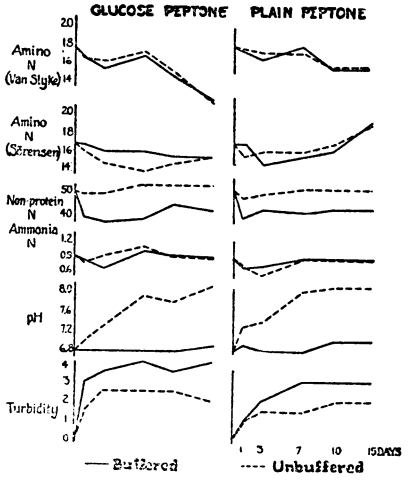

FIG. 13

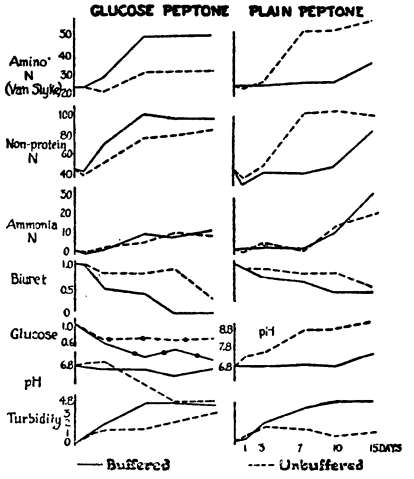

FIG. 14

FIG. 13. BACTERIUM BRONCHISEPTICUM
FIG. 14. PSEUDOMONAS PYOCYANEA (N. Y. U.)

buffered glucose peptone were quite pronounced, and approximated those occurring in the unbuffered sugar-free medium. On the other hand, in the unbuffered glucose peptone the proteolytic action of this organism was relatively small. Biuret disappeared much more rapidly in the buffered than in the unbuffered tubes, and glucose utilization was also much greater in the former.

The presence of added phosphate in the plain peptone apparently retarded the proteolytic activity of *Ps. pyocyanea*, if amino and non-protein nitrogen are taken as an index. Production of free ammonia was approximately the same in the buffered and

unbuffered glucose-free peptone. In the peptone containing no sugar the ammonia remained at or near zero until after the seventh day when there was a marked and continuous rise. The total increase, however, was greater in the buffered medium.

It is interesting to note that the added phosphate exerted a strong regulatory action on pH in both the plain and glucose peptone cultures, and encouraged greater luxuriance of growth, as indicated by turbidity.

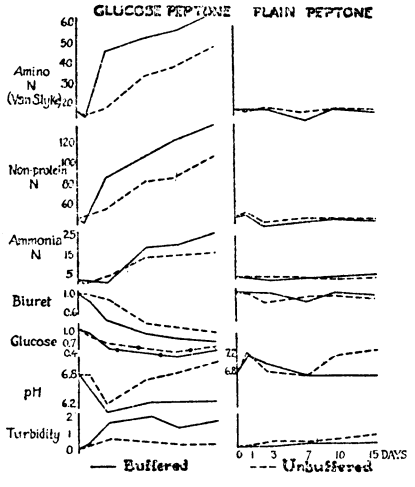

FIG. 15

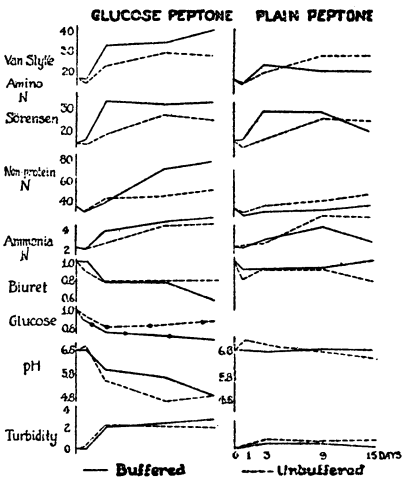

FIG. 16

FIG. 15. CLOSTRIDIUM SPOROGENES
FIG. 16. CLOSTRIDIUM WELCHII

Clostridium sporogenes

Added phosphate markedly accelerated the metabolism of this anaerobe in glucose peptone (fig. 15). The amino and non-protein nitrogen increase was not only much more rapid in the buffered glucose medium during the first three days of growth, but continued to be approximately twice that of the corresponding unbuffered glucose-containing tubes during the entire fifteen-day period of observation.

In agreement with the changes in amino and non-protein nitrogen, the biuret fraction disappeared more rapidly from the

buffered tubes than from the other. More abundant production of free ammonia also occurred in the buffered carbohydrate medium. Turbidity figures indicate that growth was much more luxuriant in the buffered glucose peptone tubes than in the unbuffered.

In the plain medium *Cl. sporogenes* gave a scanty growth, and produced no appreciable changes in the various nitrogen fractions.

Clostridium welchii

Although the nitrogen changes produced by *Cl. welchii* in buffered glucose peptone were not so extensive as those brought about by *Cl. sporogenes*, the response of this anaerobe to added phosphate was similar in nature (fig. 16). Figures for amino nitrogen and non-protein nitrogen, as well as free ammonia, were higher in the buffered glucose peptone than in the unbuffered sugar medium, and corresponding differences in biuret reduction were apparent. With this increased peptolytic activity, it is interesting to note the favorable influence of the phosphate buffer in preventing the rapid increase in H-ion concentration which took place in the unbuffered glucose peptone.

Growth in the carbohydrate-free medium was slight, showing the dependence of this organism on sugar for energy requirements. No material differences were observed in the two sugar-free media.

GENERAL DISCUSSION

Although phosphate buffer accelerated the growth and metabolic activities of many of the organisms, it did not affect all members of the same group, or even closely related species in the same manner. For example, members of the subtilis group varied in their response to added phosphate in both plain and glucose-containing peptone solutions. *B. subtilis* and *B. cereus* produced more free ammonia in the buffered sugar-free medium than in the unbuffered plain peptone, whereas *B. megatherium* formed little free ammonia in the buffered plain medium, but large amounts in the unbuffered.

B. subtilis displayed marked increases in amino and non-protein nitrogen, whether grown in plain or in glucose peptone. *B.*

cereus and *B. megatherium*, on the other hand, gave little evidence of proteolytic changes in the glucose peptone on account of the rapid increase in H-ion concentration. The greater activity *B. subtilis* in glucose peptone is without doubt due to its ability to utilize non-carbohydrate carbon for energy about as readily as that of glucose, and thus to maintain a reasonable acid-alkali balance.

E. prodigiosus resembled *B. cereus* in the nitrogenous changes produced in the unbuffered media, but it differed from *B. cereus* in the buffered glucose medium. Its peptolytic activity, as shown by the amino nitrogen, non-protein nitrogen, and biuret changes, was considerable in the buffered glucose-containing cultures, but very slight in the unbuffered sugar medium. The rapid drop from pH 6.8 to 4.8 which occurred in the unbuffered tubes of both *B. cereus* and *E. prodigiosus* explains the lack of change in the various nitrogen fractions of these cultures, while the maintenance of a more favorable pH in the buffered medium permitted considerable peptolytic action. Glucose utilization was greater in the buffered tubes of *E. prodigiosus* than in the unbuffered, as was also the case with *B. cereus* and *B. megatherium*.

In contrast to the marked peptolytic activity of the subtilis group, members of the coli-typhi-paratyphi group exhibited a relatively slight nitrogen metabolism. *Bact. pullorum* and *Bact. typhosum* resembled each other closely in their metabolic activities. A decrease in amino nitrogen was generally observed in the glucose peptone cultures, while in the sugar-free media a noticeable increase in that fraction occurred after the first three days' of growth. The buffered plain peptone cultures did not differ very greatly from the unbuffered, although there was some indication of greater proteolytic activity in the former.

Bact. coli, like *Bact. typhosum*, showed little nitrogenous change in the glucose-containing medium, but in plain peptone this organism was decidedly more active than *Bact. typhosum*. The nitrogen metabolism of *Bact. paratyphosum* A and B was in many ways similar to, but more pronounced, than that of *Bact. typhosum* and *Bact. pullorum*.

It is interesting to note that in the buffered glucose-containing

cultures of *Bact. coli*, *Bact. pullorum*, *Bact. typhosum*, and *Bact. paratyphosum* A and B, more sugar was utilized than in the unbuffered, and along with this change a more luxuriant growth and a greater decrease in free ammonia was observed. The amount of phosphate buffer added to plain peptone cultures of this group exerted a strong regulatory effect on H-ion concentration, but in the glucose medium the buffer checked the rapid drop in pH to only a small degree.

The addition of one per cent phosphate to cultures of *Proteus vulgaris* proved sufficient to maintain a favorable H-ion concentration, even in the presence of glucose, and to encourage continued nitrogen metabolism. Amino nitrogen values were as a rule higher in the buffered cultures. Free ammonia production was greater in the buffered media, irrespective of the presence or absence of glucose, and in the unbuffered glucose-peptone cultures only a slight increase took place.

Staph. aureus in glucose peptone showed a greater decrease in the amino nitrogen fraction than did the coli-typhi group. In plain peptone an initial decrease in amino nitrogen occurred, followed by an increase. Ammonia nitrogen also decreased somewhat during the entire fifteen days of growth in the glucose-containing cultures. The added phosphate checked the rapid drop in pH to a small extent.

Bact. bronchisepticum with its slight metabolic activity more or less resembles the colon group of organisms, but proved to be unique in that it showed a progressive decrease in amino nitrogen during the fifteen day observation period.

Cl. sporogenes showed a much greater increase in amino and non-protein nitrogen and in free ammonia in the buffered glucose peptone than in the unbuffered, with more luxuriant growth in the former tubes. The metabolic end products of this anaerobe were such that the total pH reduction in the glucose-containing media was small.

Cl. welchii also displayed more peptolytic activity in buffered glucose peptone than in the unbuffered. This seems to be directly associated with the regulatory effect of the added phosphate on H-ion concentration, for turbidity estimations indicated similar

amounts of growth. Amino and non-protein nitrogen, and free ammonia production proved to be decidedly greater in the buffered than in the unbuffered glucose medium.

CONCLUSIONS

Phosphate buffer when present in reasonable amount (1 per cent) will in many instances maintain a sufficiently low level of H-ion concentration to permit the given organisms to bring about nitrogenous changes in the substrate as though no utilizable carbohydrate were present.

The ability to carry on such activities varies widely with different bacterial groups or species, and even with members of the same species.

The methods employed in this investigation make it possible to study nitrogen metabolism over a wide range, as indicated by the comparative determinations of total, protein, non-protein, ammonia, amino and polypeptid nitrogen, and biuret.

Aside from the influence of buffer on nitrogen metabolism, it was hoped to make this study of considerable interest in furnishing definite information concerning the activities of 22 different, well-known species, not only as to nitrogen changes, but as to the action of these organisms on glucose, and the resultant changes in H-ion concentration under the different experimental conditions.

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