SOME REGULATING FACTORS IN BACTERIAL METABOLISM

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Kendall and his co-workers, in a series of investigations, have developed the idea that in the metabolism of bacteria sugar has a sparing effect on the digestion of protein and that, consequently, the continuous feeding of sugar to animals would induce a change in the intestinal flora from a proteolytic to an acid forming type. These results on sugar feeding were confirmed recently by Rettger working on white mice and chickens and by Torrey on human typhoid patients.

In his investigations Kendall used a constant proportion of sugar to peptone (1 per cent of each) in standard nutrient broth. Observations, made in the course of a study on the proteolytic enzymes of the *B. proteus*, in media in which the concentration of sugar was varied, led me to think, however, that there were other important factors involved in bacterial metabolism, the actual effects of which were not known.[1] It became quite evident from these and other observations that the relative concentrations of sugar and peptone were significant; that different organisms of closely related groups were capable of utilizing varying amounts of sugar, and that the amount of sugar that a particular form could completely ferment varied within limits with the concentration of the other ingredients, especially peptone and phosphate salts. The following are the results of a series of preliminary experiments to test more definitely the validity of these observations:

[1] Kendall and Walker in an independent investigation of the same problem obtained identical results.

A. Effect of different concentrations of peptone on the nitrogen utilization of certain intestinal bacteria. The best measure of the degree of bacterial metabolism in nitrogenous media is the amount of ammonia liberated. This criterion was therefore used. The technique was, briefly, as follows:

Simple solutions of peptone in distilled water containing varying amounts of peptone and 0.5 per cent glucose were used. Two series were run; one with Witte's and the other with an American peptone. The different lots of media from each peptone were made at the same time, flasked, and autoclaved under the same conditions. Inoculations were made from young cultures and the flasks incubated at 30°C. for seven days. One lot of Witte peptone was incubated for five days, but this period was found insufficient.

The ammonia determinations were made according to the Folin micro method. First, 5 cc. of the culture were used but later 10 cc. were found more satisfactory, due to the small amounts of ammonia present. The period of aeration was twenty minutes. The ammonia was caught in 10 cc. $\frac{N}{50}$ sulphuric acid and the excess acid titrated with $\frac{N}{50}$ Na(OH).

The effect of different concentrations of peptone is evident from the results of this experiment. It is, of course, possible that the greater ammonia production with the higher concentration of peptone is due merely to the favorable action of the increased amount of buffer in keeping down the hydrogen ion concentration. That the concentration of the peptone is in itself an important factor, is however indicated by the different results obtained with different types of bacteria. *B. typhi*, for instance, produced no ammonia in the 0.5 per cent peptone in one medium and a negative quantity in the other, while in the higher concentrations small amounts were obtained, though less than in the sugar-free control. *B. coli* and *B. cloacae*, etc., give increasing amounts of ammonia with the increasing concentration of peptone; but in all cases the amount still remains lower than in the sugar-free control.

The results, on the whole, indicate that the concentration of peptone is an appreciable factor when the concentration of glucose is moderately low. Attention may also be called in passing

to the different results with the two peptones. This is, no doubt, due to an essential difference in the composition of the two products. Since Rettger has shown that certain bacteria do not

TABLE 1

Amount of ammonia produced in solutions containing different amounts of Witte's peptone and 0.5 per cent sugar

ORGANISM	AMOUNT OF NH_3 IN CGM. IN 100 CC. OF MEDIA*							
	0.5% peptone 0.5% glucose		1.0% peptone 0.5% glucose		1.5% peptone 0.5% glucose		1.5% peptone No glucose	
	5 days	7 days	5 days	7 days	5 days	7 days	5 days	7 days
B. coli	−0.17	0.17	0.0	0.34		0.68	0.34	0.34
B. cloacae	0.17	0.51	0.17	0.68		0.85	1.02	0.68
B. paracoli	−0.51	0.00	0.00	0.17	0.34	0.17	0.85	0.51
B. capsulatus	0.17	0.17		0.51	0.17	0.34	0.85	0.68
B. typhi	−0.68	0.00	−0.17	0.00	0.00	0.00	0.17	0.34
Uninoculated control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

* The data presented here were obtained from two distinct sets of tests run on different occasions. In the first a five-day and in the second a seven-day period of incubation was used. The effects of the different amounts of peptone on the ammonia yield are brought out in both series.

TABLE II

Amount of ammonia produced in solutions containing different amounts of American peptone and 0.5 per cent glucose

ORGANISM	AMOUNT OF NH_3 IN CGM. IN 100 CC. OF MEDIA			
	0.5% peptone 0.5% glucose	1% peptone 0.5% glucose	1½% peptone 0.5% glucose	Control, 1% Peptone, no sugar
	7 days	7 days	7 days	7 days
B. coli	0.17	0.17	0.34	2.04
B. cloacae	0.34	0.51	0.68	2.38
B. paracoli	0.00	0.00	0.17	1.19
B. typhi	0.00	0.17	0.17	0.51
B. capsulatus	0.17	0.17	0.68	1.70
Uninoculated control	0.00	0.00	0.00	0.00

digest proteoses and peptones, it seems that the American product is very likely richer in amino-acids and hence gives a higher yield of ammonia in all instances.

B. Effect of different concentrations of peptone and glucose and phosphate on the metabolism of intestinal bacteria. Another index

of the metabolism of bacteria is the change they produce in the hydrogen ion concentration of the medium as a result of their activities. The acid titration or the ammonia determination indicates only the action on either the carbohydrates or the peptone present in the medium. The hydrogen ion concentration, on the other hand, gives the resultant of the action on both the carbohydrate and nitrogenous components. By determining the hydrogen ion concentration on different days, one may even trace the progressive increase with the active utilization of the sugar and subsequent decrease, if any occurs, during the active utilization of the constituents of the peptone. This test, furthermore, serves as a simple index of (a) the amount of sugar a particular organism may utilize without producing sufficient acid to inhibit further growth and (b) the rate at which different organisms can utilize a particular sugar. Experiments are under way which indicate that distinct and constant differences exist in both properties among closely related types.

The method with some slight modifications is that used by Clark and based on Sorensen's colorimetric method of determining the hydrogen ion concentration. Standard solutions of primary and secondary phosphate and of sodium acetate-acetic-acid and the indicators described by Clark and Lubs were used.

Media were made as above containing varying amounts of peptone, glucose and phosphate. A series of typical cultures was inoculated into these media, incubated at 30° C. and tests made at regular intervals. In order to eliminate the color, due to the breaking down of the glucose during sterilization it was found necessary to sterilize the peptone-phosphate solution and the sugar solution separately, and then add the sugar to the peptone by means of sterile pipettes. This somewhat increased the difficulty in making the medium but assured a water-clear solution, which greatly facilitated the color readings. For testing the hydrogen ion concentration 1 cc. of the culture was mixed with 5 cc. of freshly distilled water[2] and three to four drops of the indicator added.

[2] Clark has suggested a dilution of 1 : 10 but a few preliminary tests showed that it was more desirable to use a 1 : 5 dilution as excessive dilution changes the hydrogen ion concentration.

(1) *Effects of different amounts of peptone with and without the addition of phosphate.* The object of this experiment was to determine the effects of different concentrations of peptone on the carbohydrate and nitrogen utilization by bacteria and the influence exerted by the addition of an acid regulator such as primary sodium phosphate ($Na_2H\ PO_4$). The media were made as outlined above and tests for the hydrogen ion concentration run on the second and fourth days. With a little care 1 cc. of the culture can be withdrawn by means of a sterile pipette without contaminating it. By this simple method the progressive changes in the same culture tube could be followed for a week or more by daily withdrawals of 1 cc. samples.

The results of these tests are summarized in table III. They indicate quite clearly that the concentration of peptone plays a rather significant rôle in accelerating glucose fermentation, as well as in favoring its complete utilization. The former effect is seen in table III, section A, in the differences in the P_H^+ values in the *B. cloacae* cultures on the second day and in the *B. aerogenes* cultures on the fourth day. A similar effect is noted in table III, section B, in the case of *B. coli.* The favorable effect on complete utilization of the carbohydrate is brought out especially in the case of *B. aerogenes* in section A and of *B. coli* in section B of table III.

The effect of the regulating action of the acid-phosphate is evident on comparing sections A and B. By keeping down the hydrogen ion concentration of the medium some of the organisms are enabled to use up all the carbohydrate (0.5 per cent) without producing sufficient concentration of acid to inhibit their growth. Once this is accomplished, the active utilization of the nitrogenous constituents commences and we obtain a progessively decreasing hydrogen ion concentration (or an increasing P_H^+ value). That the regulating effect of the phosphate is not the only factor, however, is evident from the results obtained with *B. coli* as shown in table III.

It is interesting to note that in the absence of phosphate the cultures of *B. cloacae* and *B. aerogenes* reach a hydrogen ion concentration practically as high as that of *B. coli,* which does

not occur in the same medium when 0.5 per cent phosphate is present. Another interesting point is that the final hydrogen ion concentration is higher when no phosphate is present; the primary phosphate obviously tends to keep down the concentration of the hydrogen ions.

TABLE III

The effects of different amounts of peptone, with and without phosphate, on the utilization of the carbohydrate and peptone, as indicated by the hydrogen ion concentration

COMPOSITION OF THE MEDIA													
		A						B					
Phosphate		0.0%		0.0%		0.0%		0.5%		0.5%		0.5%	
Glucose		0.5%		0.5%		0.5%		0.5%		0.5%		0.5%	
Peptone		0.5%		1.0%		1.5%		0.5%		1.0%		1.5%	
		P^+_H		P^+_H		P^+_H		P^+_H		P^+_H		P^+_H	
Days		2	4	2	4	2	4	2	4	2	4	2	4
Cultures	B. coli*		4.8		4.6		4.6	*4.8*	*5.1*	*5.4*	*6.6*	*6.0*	*6.6*
	B. paracoli	4.6	4.8	4.6	4.8	4.8	4.6	5.0	5.2	5.2	5.2	5.3	5.0
	B. cloacae	*5.2*	*5.6*	*5.6*	*6.6*	*6.4*	*6.8*	6.5	6.8	6.6	7.0	6.8	7.2
	B. aerogenes	4.6	*4.6*	4.8	*5.0*	4.8	*5.6*	6.6	6.6	6.4	6.8		
	B. typhi	4.8	4.6	4.6	4.7	4.6	4.8	5.0	5.1	4.8	5.0		

* This strain of *B. coli* gave these results constantly. Observations in connection with other experiments indicate that not all strains behave in this manner. It is quite likely that there are two types of *B. coli* (corresponding to the *B. communior* and *B. communis* respectively), which may be differentiated in this way.

(2) *Effect of different concentrations of phosphate on the metabolism of bacteria.* The results of the experiments recorded above indicated that primary phosphate, acting apparently as an acid regulator, played an important part in controlling the nutritive processes of bacteria. In order to get more light on the nature of this regulatory mechanism and its influence on the carbohydrate and peptone utilization the following tests were performed:

Media were made containing a constant and optimum amount of peptone (1 per cent), comparatively low concentrations of

glucose (0.3 per cent and 0.4 per cent, respectively), and varying amounts of phosphate salt, (0.2 per cent, 0.3 per cent and 0.4 per cent, respectively). As nearly as possible the methods of preparation of media, inoculation, incubation, etc., were kept uniform. Tests were made on the first, third, and fourth days, respectively, with the results given in table IV.

TABLE IV

Effect of different concentrations of acid phosphate on the utilization of glucose by bacteria

	A			B		
Peptone Glucose Phosphate	1.0% 0.3% 0.2%	1.0% 0.3% 0.3%	1.0% 0.3% 0.4%	1.0% 0.4% 0.2%	1.0% 0.4% 0.3%	1.0% 0.4% 0.4%
No. of culture	P_H^+ value	P_H^+ value	P_H^+ value	P_H^+ value	P_H^+ value	P_H^+ value
11	4.9	4.9	5.0	4.9	5.0	5.1
16	*4.7*	4.8	*6.2*	4.7	4.8	5.5
17	*5.0*	5.0	*6.5*	4.6	4.6	5.0
18	*6.0*	6.4	*7.3*	*4.8*	4.6	*7.4*
19	*5.0*	*6.2*	*6.8*	*4.8*	4.7	*7.2*
22	*4.9*	5.2	*6.5*	4.8	4.8	5.0
24	6.6	6.6	7.4	6.2+	6.2+	7.4
40	6.6	6.6	*6.8*	6.2+	6.2+	6.8
44	4.9	5.0	5.4	4.8	4.9	4.9
135	*5.6*	5.8	*6.6*	*4.9*	4.7	*6.5*
196	4.8	5.4	5.6	5.4	5.5	5.2
239	*5.0*	5.0	*6.4*	4.8	4.8	5.0

To avoid the confusion that might result from unnecessary detail only the four-day tests are tabulated. The effect of the phosphate is strikingly shown in both the A and B section of the Table. The significant results are italicized. The low figures represent high hydrogen ion concentrations—or the acid phase—while the high figures represent low hydrogen ion concentrations or the alkaline phase. The progressive change produced in carbohydrate media by members of the colon-typhoid group, excepting certain types, is from the alkaline to acid and back. So long as there remains unutilized carbohydrate the acid phase persists. With the complete consumption of the

carbohydrate the organism actively attacks the nitrogenous components of the medium, neutralizing the acid and gradually returning to the alkaline phase. The phosphate evidently takes care of the free acid, thus keeping the ionic concentration below the lethal point and enabling the organism to proceed in its activity. As a result of this regulative power the amount of sugar which a particular species can completely utilize varies within limits with the relative amount of primary phosphate salts present in the medium. Thus, in the presence of only 0.2 per cent phosphate, 0.3 per cent sugar is completely digested (as indicated by the low hydrogen ion concentration) by only four organisms (18, 24, 40 and 135) while only two (24 and 40) can use up 0.4 per cent glucose. In the presence of 0.3 per cent phosphate on the other hand 0.3 per cent glucose is also broken down by number 19; and when 0.4 per cent phosphate is added all but one are capable of completely destroying 0.3 per cent sugar, while five (18, 19, 24, 40 and 135) can even use up 0.4 per cent glucose. On increasing the amount of sugar to 0.5 per cent even in the presence of 0.5 per cent phosphate only 19, 24 and 40 reach the alkaline phase (see table III).

C. Amount of sugar digested by different species. Aside from the effect of varying concentrations of phosphate, the different types manifest specific differences in their power to digest definite quantities of glucose under a given set of conditions. These differences are not attributable to the toxic action of the high hydrogen ion concentration on the cell, alone. Why one organism (11, for instance) (see table IV) should not be able to use 0.3 per cent glucose while number 18 will do away with 0.4 per cent with the same amount of phosphate (see table IV), when both reach approximately the same limiting hydrogen ion concentration (4.8 and 4.9, respectively, table IV, section B), is not evident on the surface. The difference is partly explicable on the basis that number 18 (*B. enteritidis*) carries the fermentation further than number 11 (*B. typhi*), thus removing the acid and its inhibitive effects. But this would not explain the differences between numbers 17, 18 and 19, for instance, all gas-producing forms. We must assume either that there are inhibiting factors

of a specific character or else that specific differences exist in the way the glucose molecule is split by these different forms giving rise to different amounts of toxic substances. The interesting fact remains that, under the same conditions, these closely related forms manifest strikingly different powers of utilization of sugar. Such a difference exists between *B. aerogenes* and *B. coli*, and Clark, taking advantage of this has devised a simple method for differentiating the two under a given set of conditions. The difference between *B. aerogenes* and *B. coli* is, perhaps, more fundamental than that between the other members of the group. Specific differences do exist, however, and experiments are now well under way, which promise to furnish a basis for separating certain of the other more closely related members of this group.

SUMMARY AND CONCLUSIONS

A study of some of the factors involved in the regulation of bacterial metabolism was made. The effects of different concentrations of peptone on the utilization of glucose and amino-acids, and of different concentrations of phosphate on the fermentation of glucose were studied. The nature of the metabolic changes was measured by ammonia determinations by the Folin method and by noting the change in the hydrogen ion concentration by the Sorensen-Clark method. The results were, briefly, as follows:

1. The concentration of peptone is an appreciable factor, controlling the nutrition of bacteria. With a moderate amount of glucose present the higher the concentration of peptone the greater the amount of ammonia produced. The amount of ammonia is in no case as great as in the sugar-free control, indicating a distinct sparing effect.

2. Primary phosphate acting as an acid-regulator plays a very important part in the regulating of the carbohydrate utilization by different bacteria of the colon-typhoid group.

3. The different members of the colon-typhoid group manifest specific differences in their power of completely utilizing different amounts of sugar under a given set of conditions. These differences may be utilized for species differentiation.