CXXIX. FLUORIDE SENSITIVITY OF *PROPIONIBACTERIUM PENTOSACEUM* AS A FUNCTION OF GROWTH CONDITIONS

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The isolation by Werkman *et al.* [1936] of phosphoglyceric acid from a bacterial fermentation of glucose suggested the occurrence of a dissimilation similar to that already proposed for muscle by Embden *et al.* [1933], and extended to yeast by Meyerhof & Kiessling [1934]. The formation of phosphoglyceric acid by the propionic acid bacteria as well as by many other species has been shown by Stone & Werkman [1936, 1, 2; 1937].

That the fermentation of glucose necessarily passes through the intermediate stage of phosphoglyceric acid was questioned by Werkman *et al.* [1937] when proliferating cells were found to dissimilate glucose at a concentration of NaF which inhibited the breakdown of phosphoglyceric acid. It seemed unlikely, therefore, that the dissimilation was proceeding exclusively through this ester. Werkman *et al.* [1937] found that phosphoglyceric acid was readily dissimilated. This fact was rather striking since a satisfactory dissimilation of phosphoglyceric acid by resting cells had not been previously obtained in this laboratory. Inasmuch as the work of Werkman *et al.* [1937] was carried out with proliferating cells, it appeared that significant differences existed between cells grown on phosphoglyceric acid and those obtained from glucose media. Therefore the effect of culturing the organism in the presence of phosphoglyceric acid on its ability to dissimilate this compound was determined. Furthermore, since the organism appeared able to adapt itself, it was of interest to determine whether some similar concept might explain growth in NaF, i.e. whether the organism is adaptive with respect to the path by which glucose is dissimilated. If this suggestion were true organisms grown in phosphoglyceric acid media might acquire the ability to ferment this compound and then dissimilate glucose through this ester as an intermediate. On the other hand, organisms grown in the presence of NaF might lose the ability to ferment phosphoglyceric acid and develop a mechanism dissimilating glucose through some intermediate other than phosphoglyceric acid. Since the breakdown of phosphoglyceric acid is inhibited by NaF, organisms with a "phosphoglyceric acid" glucolytic system should be sensitive to NaF, while those using some other course should be insensitive to this compound.

We have succeeded in obtaining two types of organisms differing in their sensitivity toward NaF and in their ability to ferment phosphoglyceric acid. The evidence supports the suggestion that the difference in behaviour is enzymic.

METHODS

The basal medium was 0·5% yeast extract (Difco) and 1·0 or 1·5% $NaHCO_3$ buffer. (1) The yeast extract and glucose, (2) NaF and $NaHCO_3$ and (3) Na phosphoglycerate[1] were sterilized separately by autoclaving. The $NaHCO_3$, containing bromothymol blue indicator, was saturated with CO_2 before the other ingredients were added and the flask inoculated from a glucose broth culture of *Propionibacterium pentosaceum* (49 W). Flasks were incubated at 30°; CO_2 was bubbled through the medium during the entire 6·5 day incubation period. The cells were centrifuged, resuspended in 25 volumes of distilled water, allowed to stand 15 min. and again centrifuged, usually for 1 hr. or more. After decanting the wash water, the cells were resuspended in 25 volumes of 0·05 *M* K phosphate buffer (*p*H 6·3). The suspensions were tested under N_2 for ability to ferment phosphoglyceric acid and for NaF sensitivity by measuring the CO_2 produced in Barcroft-Warburg manometers.

EXPERIMENTAL

Typical data are presented in Table I. Cells were grown in three flasks, the first containing in addition to the basal medium, 0·8% Na phosphoglycerate and 0·1% glucose, the second 0·02 *M* NaF and 0·5% glucose and the third 0·5% glucose only. The cells grown in the presence of phosphoglyceric acid were quite active in dissimilating this ester (cup 1), and behaved as though phosphoglyceric acid were an intermediate inasmuch as the fermentation of glucose was entirely inhibited by 0·02 *M* NaF (cup 2).

Cells grown in NaF and glucose, however, showed a different behaviour. The dissimilation of phosphoglyceric acid was negligible (cup 7), while the gas production from glucose in the presence of NaF (cup 8) was almost as great as in its absence (cup 9). In the majority of our experiments these NaF organisms actually formed more CO_2 in the presence of NaF than in its absence. We shall return to this point. Organisms grown in NaF behaved as organisms using an intermediate mechanism not sensitive to this poison.

It is interesting that the organisms grown in glucose without NaF were quite similar to those grown in phosphoglyceric acid. They dissimilated phosphoglyceric acid readily (cup 12) and were fluoride-sensitive with respect to glucose dissimilation (cups 13 and 14). It might appear that the original supposition that the organisms must be grown on phosphoglyceric acid in order to dissimilate the ester was not true, for the organisms grown on glucose were quite similar to those grown on phosphoglyceric acid. However, an experiment is described later which correlates the fermentation of phosphoglyceric acid and growth on this ester. The observation that phosphoglyceric acid-fermenting, NaF-sensitive organisms can be obtained from glucose simplified further experimentation and obviated the use of phosphoglyceric acid in growing such organisms.

The results described suggest that *Propionibacterium* can dissimilate glucose by at least two paths. By one path, probably involving phosphoglyceric acid, the organism is NaF-sensitive, whereas by the other path (or paths) it is not sensitive. Presumably the latter route does not involve phosphoglyceric acid. It appears that organisms of either type may be obtained by introducing or omitting the poison in the medium.

[1] Kindly provided by Prof. Carl Neuberg as barium phosphoglycerate.

Table I. *Dissimilation of glucose by cells grown in the presence and absence of NaF*

Basal medium plus ...	Cells grown in glucose 0·1%, phosphoglyceric acid 0·8%				
Cup no.	1	2	4	5	6
Main chamber ...	1·7 ml. of organisms in each cup (under nitrogen)				
Side cup	0·2 ml. Pga, 0·1 ml. H_2O	0·2 ml. G, 0·1 ml. NaF	0·2 ml. G, 0·1 ml. H_2O	0·3 ml. H_2O	0·1 ml. NaF, 0·2 ml. H_2O
μl. CO_2 evolved					
120 min.	65·7	3·5	96·2	1·5	6·1
310 min.	143·0	2·3	128·7	3·0	6·1
490 min.	152·0	2·3	140·0	1·5	6·1
490 min. with control subtracted	150·5	−3·8	138·5	—	—
Basal medium plus ...	Cells grown in glucose 0·5% NaF 0·02 *M*				
Cup no.	7	8	9	10	11
Main chamber	1·7 ml. of organisms in each cup (under nitrogen)				
Side cup	0·2 ml. Pga, 0·1 ml. H_2O	0·2 ml. G, 0·1 ml. NaF	0·2 ml. G, 0·1 ml. H_2O	0·3 ml. H_2O	0·1 ml. NaF, 0·2 ml. H_2O
μl. CO_2 evolved					
120 min.	9·1	83·9	94·9	12·8	14·5
310 min.	22·1	129·7	143·0	19·9	31·9
490 min.	31·2	141·1	156·8	24·2	37·7
490 min. with control subtracted	7·0	103·4	132·6	—	—
Basal medium plus ...	Cells grown in glucose 0·5%				
Cup no.	12	13	14	15	16
Main chamber	1·7 ml. of organisms in each cup (under nitrogen)				
Side cup	0·2 ml. Pga, 0·1 ml. H_2O	0·2 ml. G, 0·1 ml. NaF	0·2 ml. G, 0·1 ml. H_2O	0·3 ml. H O	0·1 ml. NaF, 0·2 ml. H_2O
μl. CO_2 evolved					
120 min.	52·5	22·9	120·0	−2·8	+13·4
310 min.	140·1	31·5	177·9	−11·3	11·9
490 min.	172·2	37·2	190·3	−14·3	13·4
490 min. with control subtracted	184·5	23·8	204·6	—	—

Pga = phosphoglyceric acid 0·25% (0·135 *M*) (86·5 mg. BaPga + 40 mg. Na_2SO_4 + 1·8 ml. H_2O, filter; make up to 2 ml.).
G (glucose) = 0·125% (0·069 *M*).
NaF = 0·4 *M*.
Organisms = 4% by volume in 0·05 *M* phosphate buffer (*p*H 6·3).

In Table II the cells were harvested after 4 days' growth instead of the usual 6·5 days. One condition, in addition to time, was unavoidably different in this experiment and for this reason it is difficult to say that the somewhat different results are due entirely to a shorter growth period. The yeast extract used was in both cases "Difco," but that used in Exp. 1 was considerably lighter in colour, indicating differences in composition. The experiment is described here because it shows that the ability of micro-organisms to dissimilate phosphoglyceric acid is affected by growing them in the presence of the acid. The dissimilation of phosphoglyceric acid (cup 1) is rapid when compared with that of organisms grown on NaF and glucose, and glucose (cups 7 and 13 respectively). The greater fermentation of phosphoglyceric acid by "fluoride" organisms than by "glucose" organisms is not in agreement with expectation. In this connexion, it is interesting that the total CO_2 formed is about the same (cups 7 and 13) and the difference becomes apparent when the controls (cups 12 and 18) are subtracted. The NaF sensitivity of cells grown on phosphoglyceric acid does not

Table II. *Dissimilation by cells grown in various media*

Basal medium plus ...	Cells grown in glucose 0·1%, phosphoglyceric acid 0·8%					
Cup no.	1	2	3	NaF inhibition %	5	6
Main chamber	1·7 ml. of organisms in each cup					
Side cup	0·2 ml. Pga, 0·1 ml. H_2O	0·2 ml. G, 0·1 ml. NaF	0·2 ml. G, 0·1 ml. H_2O	—	0·2 ml. H_2O, 0·1 ml. NaF	0·3 ml. H_2O
μl. CO_2* evolved						
30 min.	25·8	12·4	40·6	69·5	1·5	−1·2
60 min.	34·1	25·0	71·5	72·0	2·9	−1·2
105 min.	50·3	44·4	100·7	52·6	4·4	+0·6
195 min.	83·8	76·8	124·7	38·5	5·8	+1·2
330 min.	134	114·8	135·7	15·4	13·1	+8·5
765 min.	170	145·5	151·7	4·1†	16·1	+12·2
765 min. with control subtracted	157·8	—	—	—	—	—
Basal medium plus ...	Cells grown in glucose 0·5%, NaF 0·02 *M*					
Cup no.	7	8	9	NaF inhibition %	11	12
Main chamber	1·7 ml. of organism in each cup					
Side cup	0·2 ml. Pga, 0·1 ml. H_2O	0·2 ml. G, 0·1 ml. NaF	0·2 ml. G, 0·1 ml. H_2O	—	0·2 ml. H_2O, 0·1 ml. NaF	0·3 ml. H O
μl. CO_2* evolved						
30 min.	16·9	38·3	30·1	−27·5	4·3	1·5
60 min.	18·2	72·0	59·0	−22·0	8·7	2·9
105 min.	23·4	122·1	104·2	−17·1	14·5	5·8
195 min.	32·4	133·6	123·2	−8·4	24·6	8·8
330 min.	55·8	173·6	140·5	−73·6	40·6	19·0
765 min.	72·0	197·7	151·2	−30·7	50·8	24·8
765 min. with control subtracted	47·2	—	—	—	—	—
Basal medium plus ...	Cells grown in glucose 0·5%					
Cup no.	13	14	15	NaF inhibition %	17	18
Main chamber	1·7 ml. of organisms in each cup					
Side cup	0·2 ml. Pga, 0·1 ml. H_2O	0·2 ml. G, 0·1 ml. NaF	0·2 ml. G, 0·1 ml. H_2O	—	0·2 ml. H_2O, 0·1 ml. NaF	0·3 ml. H_2O
μl. CO_2* evolved						
30 min.	11·5	11·1	25·9	57·1	11·0	8·3
60 min.	15·8	24·9	60·3	58·7	16·5	13·9
105 min.	21·5	48·2	87·3	44·8	22·1	16·6
195 min.	31·5	81·4	127·2	36·0	37·2	18·0
330 min.	50·1	112·7	127·8	11·8	52·4	45·9
765 min.	65·9	122·8	143·6	14·5	68·9	51·4
765 min. with control subtracted	14·5	—	—	—	—	—

Solutions and organism as in Table I.

* Controls have been subtracted in cups 2, 3, 8, 9, 14 and 15.

† The comparison after a few hr. is not quantitatively accurate, because the substrate concentration is no longer the same.

differ greatly from that of cells grown on glucose, but culturing in phosphoglyceric acid does have some effect, the cells being about 14% more sensitive.

Neither the "phosphoglyceric acid" nor "glucose" cells of this experiment are as sensitive to NaF as are those of Exp. 1. Since the growing time was less, there is the possibility that as the growing time increases a NaF-insensitive system is replaced by a NaF-sensitive one and that in the case in question this change has not yet been completed.

The point is that organisms may more rapidly acquire the ability to break down phosphoglyceric acid when grown on this ester than on glucose.

Complete inhibition of dissimilation

By measuring the anaerobic CO_2 production of *P. pentosaceum* only a small part of the total products is determined. It seemed possible that the variation observed between the two types of organisms might lie in the CO_2 formation and not apply to the other products common to the propionic fermentation.

Since the products of the propionic acid fermentation are entirely acids and CO_2, this point can be tested, using the respirometer, by suspending the organisms in $NaHCO_3$ buffer. The technique was that used by Fromageot & Chaix [1937].

The two types of organisms, grown 6·5 days on glucose and on glucose plus NaF, were washed with distilled water as previously and suspended in a buffer containing 0·20 % $NaHCO_3$ and 0·9 % NaCl. Substances to be added were dissolved in the same buffer. After saturation with CO_2 which had been passed over hot copper gauze, the suspension and solutions were placed in the manometer cups. The air in the manometer cups was then displaced by O_2-free CO_2.

The results (Table III) show that NaF entirely inhibited both acid and CO_2 formation by organisms grown in glucose. On the other hand, acid and CO_2

Table III. *NaF sensitivity of* Propionibacterium pentosaceum *as a function of growth conditions*

Basal medium plus ...	Cells grown in glucose 0·5 %				
Cup no.	1	2	3	4	5
Main chamber	1·7 ml. of organisms in each cup				
Side cup	0·4 ml. Pga	0·2 ml. G, 0·1 ml. NaF	0·2 ml. G, 0·1 ml. buffer	0·2 ml. buffer, 0·1 ml. NaF	0·3 ml. buffer
μl. CO_2* evolved					
30 min.	−4·8	+14·1	112·6	8·6	1·4
80 min.	−8·9	22·4	452·9	15·7	+1·3
230 min.	+27·8	+18·4	548·3	17·8	−6·4
470 min.	116·6	+38·0	575·8	36·4	+15·4
950 min.	229	+56	626·7	51·4	+33·8
950 min. with control subtracted	195	+4·6	592·9	—	—
Basal medium plus ...	Cells grown in glucose 0·5 %, NaF 0·02 *M*				
Cup no.	6	7	8	9	10
Main chamber	1·7 ml. of organisms in each cup				
Side cup	0·4 ml. Pga	0·2 ml. G, 0·1 ml. NaF	0·2 ml. G, 0·1 ml. buffer	0·2 ml. buffer, 0·1 ml. NaF	0·3 ml. buffer
μl. CO_2* evolved					
30 min.	+10·4	38·4	106·8	6·9	−0·2
80 min.	+12·5	247·5	417·9	12·3	+2·5
230 min.	+21·3	593·3	453·3	13·7	−2·7
470 min.	35·1	673·1	484·4	32·9	+15·7
950 min.	62·6	733·6	532·3	46·5	24·0
950 min. with control subtracted	38·6	687·1	508·3	—	—

Pga = 0·125 % in $NaHCO_3$-NaCl buffer.
G (glucose) + NaF: same as in Table I but made up in NaCl-$NaHCO_3$ buffer.
* Corrections have been made for small spontaneous evolution of gas from buffer, and increase of CO_2 solubility due to addition of Pga solution to cups 1 and 6. This disturbance probably explains retarded gas evolution in cup 1.

productions by organisms grown in the presence of NaF were only slightly inhibited by the poison in the earlier stages of the dissimilation and in the later stages the CO_2 evolution increased in the presence of NaF to a value which was higher than in its absence. The entire dissimilation is affected by the NaF, and not only the CO_2 production.

Permeability of bacterial cells to NaF

The difference in ability of the two types to ferment phosphoglyceric acid is apparently not due to the presence of NaF inside the cells grown in the poison, as might be the case if the fluoride was not thoroughly washed from the cells.

Dilution of the cells with phosphate buffer reduces the concentration of the NaF to 0·0008 *M*; moreover, washing with distilled water should remove the greater part of the fluoride before the dilution occurs. Furthermore, soaking the cells overnight in distilled water at 12° did not increase the phosphoglyceric fermentation above that of cells washed in the ordinary manner, as would be expected if some poison left in the cells of the "sodium fluoride" organisms was responsible for the small phosphoglyceric fermentation. It therefore appears that the cells are enzymically different. On the other hand, if we assume that the fluoride is not washed from the cells, we are led to the same general conclusion that the cells are enzymically different, for the dissimilation of glucose would then be proceeding in the presence of NaF contrasting with the fluoride-sensitive type.

Table IV. *Permeability experiments*

Basal medium ...	Cells under N_2 during soaking period					
Cup no.	1	2	3	4	5	6
In main chamber with 0·16 ml. of cells during 7·5 hr. soaking period	0·1 ml. H_2O	0·1 ml. H_2O	0·1 ml. NaF	0·1 ml. H_2O	0·1 ml. NaF	0·1 ml. H_2O
Side cup contents tipped in after soaking period	0·2 ml. G	0·2 ml. G	0·2 ml. G	0·3 ml. H_2O	0·3 ml. H_2O	0·1 ml. NaF, 0·2 ml. H_2O
μl. CO_2 evolved						
35 min.	51·6	38·4	43·6	7·1	2·9	6·1
275 min.	200·0	147·0	222·0	31·2	35·0	34·2
780 min.	313·0	255	342	103·0	110·0	102·0
780 min. with control subtracted	211·0	152·0	232·0	—	—	—
Basal medium ...	Cells under air during soaking period; N_2 before tipping					
Cup no.	7	8	9	10	11	12
In main chamber with 0·16 ml. of cells during 7·5 hr. soaking period	0·1 ml. H_2O	0·1 ml. H_2O	0·1 ml. NaF	0·1 ml. H_2O	0·1 ml. NaF	0·1 ml. H_2O
Side cup contents tipped in after soaking period	0·2 ml. G, 0·1 ml. NaF	0·2 ml. G, 0·1 ml. H_2O	0·2 ml. G, 0·1 ml. H_2O	0·3 ml. H_2O	0·3 ml. H_2O	0·1 ml. NaF, 0·2 ml. H_2O
μl. CO_2 evolved						
35 min.	50·6	34·4	45·4	11·4	11·6	12·7
275 min.	205·0	135·8	223·0	45·5	56·6	52·6
780 min.	323·0	226·0	338·2	126·8	121·8	132·8
780 min. with control subtracted	190·2	99·2	216·4	—	—	—

Solutions and organism as in Table I.

To investigate the possibility that cells grown in the presence of NaF are enzymically the same as those grown in glucose alone but differ only in permeability, experiments similar to those of Rünnström *et al.* [1937] were carried out. Working with bottom yeast, these investigators found a relationship between respiration and permeability. Under anaerobic conditions fermentation was inhibited by NaF; aerobically, respiration was affected only when the cells were soaked in NaF previous to glucose addition, and then inhibition was not complete. With cells in which the reserve had been depleted, however, a short period of soaking aerobically in NaF before glucose was added produced an almost complete inhibition of respiration. It was therefore suggested that impermeability was associated with respiration, the cells being less permeable when respiration was proceeding, and quite permeable under anaerobic conditions. Since all our experiments were carried out under N_2 or CO_2, the bacterial cells would be permeable to NaF. However, even if the cells are relatively impermeable, one might expect that on soaking in NaF aerobically and anaerobically for a considerable period before introducing the glucose there should occur some penetration and hence some inhibition.

As is seen in Table IV, however, 7 hr. soaking in NaF both anaerobically and aerobically before introducing the glucose caused no inhibition at all. In fact when the cells were soaked in NaF before glucose was added (cups 3 and 9) the CO_2 evolution was of the same order as when NaF was added with the glucose (cups 1 and 7) and in all of these, evolution was greater when NaF was added (cups 2 and 8). These results indicate that the NaF insensitivity of these cells is enzymic in nature, and not due to a permeability factor.

Stimulating effect of NaF on CO_2 evolution

It is not clear why the presence of NaF should cause an increase in CO_2 evolution. The effect was frequently noticed in the absence of glucose (Table II, cups 5 and 6, 11 and 12, 17 and 18; Table III, cups 4 and 5, 9 and 10) but was not as pronounced as in the presence of glucose (Table II, cups 8 and 9; Table III, cups 1 or 3 and 2, 7 or 9 and 2; Table IV, cups 7 and 8). The increase was not due to the effect of NaF on the solubility of CO_2 inasmuch as the addition of NaF to $NaHCO_3$–NaCl solution saturated with CO_2 did not measurably increase the small spontaneous evolution of gas.

Needham & Lehmann [1937, 2] mentioned increased autoglycolysis caused by NaF. However, since the effect seems to be greater in the presence of glucose we cannot ascribe it entirely to increased autoglycolysis unless the presence of glucose stimulates the endogenous metabolism. A greater glycolysis with two substrates than the sum of each has been reported by Haarmann [1932] working with vertebrate tissues.

However, NaF produces stimulation, its effects appear to be enzymic in character, and therefore to be additional evidence that NaF penetrates to the enzyme centres.

Purity of the culture

The organisms obtained from glucose and from glucose plus NaF medium were alike to all appearances. Werkman *et al.* [1937] showed that the fermentation occurring in the presence of NaF was a normal propionic fermentation. The proportion of CO_2 liberated was of the order to be expected from the propionic fermentation as was also the production of acid and CO_2 indicated by fermentation in $NaHCO_3$ buffer (Table III). Morphologically and in staining reactions the cells grown in the absence and presence of NaF were alike and characteristic of the species.

Discussion

Evidence for the existence of more than one mechanism of glycolysis in animal tissue is not wanting. Ashford & Holmes [1929] have shown that separate mechanisms exist for glucose and glycogen in brain tissue, and the work of Bumm & Fehrenbach [1931] shows that separate systems attack glucose and glycogen in white muscle. Furthermore, according to these investigators a marked substrate preference occurs among various tissues, and those which act preferentially on glucose are activated by a different coenzyme from that of the systems which act upon glycogen. A non-phosphorylating mechanism has been suggested by Needham & Lehmann [1937, 1] for embryo glucolysis.

Two mechanisms of dissimilation by the propionic acid bacteria were suggested by Wood *et al.* [1937]. Later Werkman *et al.* [1937] showed that the dissimilation of glucose by growing propionic acid bacteria was not inhibited by NaF and suggested therefore that the mechanism not involving phosphoglyceric acid was the more active.

In the present communication it has been shown that the organisms are apparently adaptable in the way in which they dissimilate glucose. These results may explain why an organism produces quantities of phosphoglyceric acid, and yet grows in the presence of NaF, and also the variable results obtained when attempting to isolate the ester.

It should perhaps be emphasized that the suggestion that only one mechanism proceeds through phosphoglyceric acid does not imply that only one route is a phosphorylating one. NaF is not necessarily a specific inhibitor for phosphoglyceric acid. It is logical to assume, however, that in this case the fluoride inhibits phosphoglyceric acid dissimilation, for it has been shown that the organism in question produces this compound.

A remark regarding the significance of phosphoglyceric acid dissimilation is pertinent. A compound which is an intermediary will break down at least as rapidly as the original substrate. In the experiments described above the dissimilation of phosphoglyceric acid by the NaF-sensitive type of organisms is only half as great as that of glucose. However, the function of phosphoglyceric acid as an intermediary according to the Embden-Meyerhof-Parnas scheme is not to arise and then break down by dismutation. Its occurrence by oxidation of triosephosphate is followed by intramolecular rearrangements to phosphopyruvic acid whose dephosphorylation is facilitated by the simultaneous phosphorylation of carbohydrate (through adenylic acid as a phosphate carrier). The pyruvic acid, or in yeast the acetaldehyde arising from it, reacts with the triosephosphate which is constantly being formed. Thus the speed of dissimilation of phosphoglyceric acid cannot be compared with that of glucose.

Summary

It has been shown that two types of cells of *Propionibacterium pentosaceum* differing in NaF sensitivity and ability to ferment phosphoglyceric acid, result from culturing in the presence and absence of NaF.

Evidence suggests that the differences are enzymic and it therefore appears that an organism may develop more than one mechanism of dissimilating glucose, depending upon environmental conditions.

The results obtained may be due to training by which a few organisms grow and multiply more rapidly than the others, thus leading to a culture able to bring about the dissimilation of the substrate in question. On the other hand

adaptation of the specific cells may occur as with galactozymase in yeast [Stephenson & Yudkin, 1936]. The present work was not designed to determine this point.

The phosphoglyceric enzyme system described here is always present; thus it is constitutive in the sense of Virtanen & Karström [Karström, 1936].

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