A STUDY OF *d*-ARABINOSE FERMENTATION

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A comparison of the fermentation of the *d*- and *l*-forms of arabinose has shown that the synthetic *d*-form was fermented slowly and with apparent difficulty by many bacteria which attacked the naturally-occurring *l*-form of the sugar quite promptly (Koser and Saunders, 1932). The results obtained with a number of typical cultures when grown in *d*-arabinose broth at 37°C. are given in table 1. Usually 3 to 10 days were required for fermentation, the time varying with the organism used. Considerable difference was apparent between various strains of the same species. Also in repeated tests with the same culture the time was found to vary somewhat, although within narrower limits. *Proteus* was the only type which was able to bring about a moderately prompt splitting of the sugar. *Proteus* and *Salmonella cholerae-suis* were unusual in that they could effect fermentation of the *d*- but not the *l*-form of arabinose. All of the other types listed in the table fermented the *l*-form promptly. Other organisms, such as staphylococci, streptococci, pneumococci, many of the *Bacillaceae* and yeasts, were unable to ferment either *d*- or *l*-arabinose.

The slow or delayed fermentations which are encountered now and then in bacteriological work have received more attention and study recently, although the explanation of the phenomenon remains largely a matter of conjecture. The late fermentation of lactose by occasional coli-like organisms and the similar slow splitting of lactose and sucrose by certain dysentery bacilli are the best known examples of this sort. In the present report we

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record some observations upon the delayed fermentation of *d*-arabinose.

EFFECT OF DIFFERENT CONDITIONS OF CULTIVATION

Concentration of sugar. The effect of increased amounts of *d*-arabinose was studied. A concentrated solution of the sugar was sterilized by filtration and added to broth to give final concentrations of 0.5, 2.0 and 5.0 per cent. The peptone and meat extract of the broth were adjusted to give concentrations of

TABLE 1

The delayed fermentation of d-arabinose

CULTURES	TIME REQUIRED FOR FERMENTATION	CULTURES	TIME REQUIRED FOR FERMENTATION
	days		*days*
Escherichia coli 1	7 to 8	*S. enteritidis* 716	3
E. coli 5	2 to 3	*S. enteritidis* 53	4 to 5
E. coli 18	2 to 3	*S. dysenteriae*, Sonne 268	8
E. aerogenes 2	5 to 7	*S. dysenteriae*, Sonne B	8 to 10
Aerobacter aerogenes 20	9 to 11	*S. cholerae-suis* 666	8 to 9
A. aerogenes M4	7 to 10	*S. cholerae-suis* 667	8 to 9
Klebsiella friedländeri	3 to 4	*S. cholerae-suis* 696	5 to 6
Salmonella schottmülleri 47	8	*S. cholerae-suis* 697	5 to 7
S. schottmülleri 822	5 to 6	*Proteus*, 3 strains	1 to 2

All of the above cultures fermented *l*-arabinose within 24 hours, with the exception of the *S. cholerae-suis* and *Proteus* strains.

Both acid and gas were produced from *d*-arabinose by all of the cultures capable of producing gas from other sugars, with the exception of *S. cholerae-suis*. These strains often produced acid only, or acid with but a small amount of gas.

1.0 and 0.3 per cent, respectively, after addition of the sugar solution. Cultures were inoculated in equal amounts into each lot of medium, incubated at 37°C. and observed closely over a period of two weeks.

With the exception of *Aerobacter aerogenes*, there was very little difference in the time required for fermentation in the several concentrations of sugar. The strains of *Escherichia coli* and representatives of the paratyphoid and dysentery groups produced acid as readily in the 0.5 per cent as in the 5.0 per cent

concentration. In fact, the fermentation appeared at times a trifle earlier in the 0.5 and 2.0 per cent tubes. Several strains of *A. aerogenes*, however, were consistently different in that acid first appeared in the 5.0 per cent concentration and later in the lower concentrations. These results are of interest when compared with the reports of others on the delayed fermentation of lactose by members of the colon group and allied types. Bronfenbrenner and Davis (1918) and Kriebel (1934) have reported that increased concentrations of lactose served to shorten the period required for fermentation.

Oxygen supply. Several reports indicate that slow fermentation of lactose may be accelerated by the increased aeration resulting from a larger surface area in relation to volume of culture. Thus Kennedy, Cummings, and Morrow (1932) observed a shortening of the time necessary to produce acidity in lactose broth when their cultures were grown in toxin flasks. Similar results have been reported by Dulaney and Michelson (1935) who found that the delayed fermentation of lactose by a coli-like organism was accelerated and that "red," or lactose-fermenting, variants appeared earlier in shallow layers of medium. On the other hand, Hershey and Bronfenbrenner (1936) stated that the proportion of fermenting variants in lactose-peptone broth increased more rapidly under conditions of partial anaerobiosis.

In our work, 5 cc. amounts of *d*-arabinose broth containing brom-cresol-purple were distributed into: (*a*) 50 cc. Erlenmeyer flasks, (*b*) ordinary test tubes of 14 mm. inside diameter, and (*c*) narrow test tubes of 10 mm. diameter. In one series of tests 0.5 per cent of sugar was employed and in another 2.0 per cent. Representative cultures of the colon, paratyphoid, and dysentery groups were then inoculated into each of the three types of containers and incubated at 37°C. Relatively little difference was apparent in the time necessary for acid production and the results secured with the narrow tubes and with the flasks were not strikingly different from those seen in the ordinary test tubes. In the shallow layers of medium in flasks there appeared to be an increased production of alkaline products. The acidity following decomposition of 0.5 per cent of *d*-arabinose did not result in as

great a change in pH value as that in the other types of containers. Also the increase in hydrogen-ion concentration under these conditions was quickly followed by a return to the neutral or alkaline range. However, when 2.0 per cent of the sugar was supplied the resulting acidity was more pronounced.

Anaerobiosis. Cultures in one-per-cent *d*-arabinose broth were placed in a jar immediately after inoculation and the usual anaerobic conditions secured by vacuum and the action of pyrogallic acid and sodium carbonate solution. Another series was prepared in which the cultures were inoculated into similar medium under vaseline seal. Under these conditions the delayed fermentation of *d*-arabinose occurred in the usual manner and showed no striking difference from the results obtained with the aerobic incubation in ordinary test tubes.

Addition of d-arabinose to older broth cultures. If sterile *d*-arabinose solution is added to nutrient broth cultures after the cultures have aged for a time equivalent to that required for fermentation, will fermentation of the sugar then be brought about rapidly? In other words, is there any change in the cells while aging in broth which would enable them to make use of the sugar promptly when it is supplied to them later, or must the cells be in contact with the sugar for a time in order to effect the readjustments necessary for its utilization? It is usually assumed that the latter is the correct explanation, although an examination of the literature dealing with delayed fermentation (usually of lactose) has revealed little information upon this point.

Cultures were grown at 37°C. in nutrient broth containing an indicator. Later, sterile *d*-arabinose solution was added to give a concentration of 0.5 per cent. The interval before addition of the sugar to the broth cultures corresponded with the period of delayed fermentation as previously determined for each culture. After addition of the *d*-arabinose solution, the cultures were held at 37°C. and observed daily for evidence of fermentation.

The results were on the whole quite uniform and in every case an additional interval was required before acid or acid and gas appeared. In many cases this interval coincided very closely

with the time required for delayed fermentation when the cultures were grown in the sugar broth medium in the usual manner.

d-Arabinose in synthetic medium. It seemed of interest to determine whether the cultures would be able to develop in a synthetic medium with the *d*-form of the sugar supplied as the only carbon compound. In ordinary nutrient broth containing *d*-arabinose the cells are supplied with numerous available foodstuffs and are not forced to use the sugar in order to attain luxuriant growth. The splitting of the sugar becomes apparent later. In a synthetic medium, however, the sugar could be made to supply the only source of energy and of carbon for structural purposes.

The synthetic medium which was employed consisted of 0.25 per cent ammonium hydrogen phosphates balanced to give pH 6.9, 0.1 per cent potassium chloride, 0.5 per cent sodium chloride, and 0.01 per cent magnesium sulphate. This was tubed in 5 cc. quantities and sterilized in the autoclave. Sterile sugar solution was added to give 0.5 per cent concentration. Two series of tubes were prepared, one containing *d*-arabinose and the other *l*-arabinose as a control. For the tests only those organisms were used which were known to be able to develop in a synthetic medium containing an ammonium salt as the only source of nitrogen and an available sugar as the source of energy.

In the control tubes of synthetic medium containing *l*-arabinose, all strains of *E. coli*, *A. aerogenes*, *Klebsiella friedländeri*, *S. schottmülleri* and *S. enteritidis* developed readily. This would be expected, since these types can utilize this sugar promptly. In contrast to this, most of these organisms failed to develop in the *d*-arabinose synthetic medium. Only one strain of *E. coli* and one of the Friedländer bacillus showed any evidence of multiplication and utilization of the *d*-form of the sugar. The growth in these cases was delayed and scanty. Although growth did not develop in the other tubes, viable cells, as determined by inoculation of agar slants, persisted for some time. Evidently when *d*-arabinose is supplied as the only source of energy, relatively few of the inoculated cells are able to effect the changes necessary

for its utilization and therefore only an occasional culture will develop.

DEVELOPMENT OF STRAINS WHICH FERMENT *d*-ARABINOSE PROMPTLY

Serial transfers in d-arabinose broth. Cultures were inoculated into broth containing 0.5 or 1.0 per cent of *d*-arabinose and an indicator. They were held at 37°C. and observed daily. Transfers were made by the ordinary wire loop to another tube of the same medium as soon as distinct fermentation became apparent. Altogether 26 cultures were carried through serial transplants in this manner.

TABLE 2

Effect of serial transfers in accelerating the slow fermentation of d-arabinose

CULTURES	DAYS REQUIRED FOR DISTINCT FERMENTATION IN EACH SUCCESSIVE TRANSFER
E. coli 1	8, 5, 5, 2, 2, 1+, 1+, 1, 1, 1
A. aerogenes 20	9, 4, 2, 2, 3, 2, 2, 2, 1+, 1, 1, 1
K. friedländeri	4, 1, 1, 1
S. schottmülleri 47	8, 6, 6, 2, 2, 3, 2, 2, 2, 2, 2, 2, 2, 1, 2, 1, 1, 1
S. dysenteriae, Sonne	9, 2, 2, 4, 2, 2, 2, 2, 1, 1, 1
S. cholerae-suis 666	9, 6, 5, 5, 4, 3, 3, 3, 3, 4, 2, 2, 2, 2, 1+ and twenty additional transfers without change

The method proved quite successful in shortening the interval required for fermentation and in most cases it was possible to obtain cultures capable of producing acid, or acid and gas, within twenty-four hours. There was considerable irregularity in the readiness with which the various cultures responded to this manipulation. Some attained this ability after only two or three serial transfers while others required many transfers before an equally prompt utilization of the sugar was evidenced. The behavior of a few representative cultures is shown in table 2.

Cultures of the various organisms which fermented *d*-arabinose promptly were plated directly from the serial transfers onto *d*-arabinose agar containing brom-cresol-purple. Of the resultant colonies, often only a small proportion appeared yellow (acid)

within the first 24 hours while the remainder showed no evidence of acid production at this time. Frequently the proportion of acid-producing colonies was less than 10 per cent of the total and it rarely exceeded 25 per cent. After 2 or 3 days, however, many more of the colonies showed evidence of fermentation of the sugar. Transfers made from the yellow colonies to *d*-arabinose broth gave rise to prompt fermentation, while similar transfers from the bluish-white or non-acid colonies required a longer interval to accomplish splitting of the sugar.

These results show that even though the broth cultures exhibited prompt fermentation they contained cells which varied considerably in their ability to attack the sugar, at least insofar as one may judge from the behavior of the resultant colonies on agar plates. Evidently the serial transfers of fermenting cultures did not eliminate cells which fermented the sugar slowly. There is also the possibility that cells which have acquired the property of speedy fermentation do not uniformly give rise to speedily-fermenting offspring, so that the resulting culture would be mixed with respect to this characteristic.

When cultures of rapidly-fermenting variants were desired for further work they were obtained by serial transfers in *d*-arabinose broth followed by immediate plating on *d*-arabinose-indicator-agar. Colonies which first showed acid production were fished and retested in the sugar broth. This process of enrichment and reisolation of the speediest fermenters was usually repeated several times. As a result cultures were secured which appeared to consist largely or entirely of cells capable of producing prompt fermentation of the *d*-form of the sugar. These strains presented a decided contrast to the original stock cultures from which they had been derived.

Daughter colonies on d-arabinose agar plates. Rapidly fermenting strains could also be obtained from the papillae which appeared in older colonies on *d*-arabinose agar. This phenomenon resembled in general that encountered with the "*E. coli-mutabile*" strains on lactose agar.

When cultures of the coli-aerogenes, paratyphoid or Sonne dysentery types were streaked over plates of nutrient agar con-

taining 0.5 or 1.0 per cent of *d*-arabinose and brom-cresol-purple or other suitable indicator, all colonies were at first white or bluish white in color. When incubation was continued and precautions taken to prevent excessive drying of the agar, small yellowish brown papillae appeared within the colonies. In some cultures these papillae appeared after only a few days, while in others they did not become evident until 10 to 14 days later. At first a few small papillae appeared and they could be detected only with the aid of a hand lens or microscope. Upon further incubation they usually became definitely larger and more numerous until most of the colonies were literally pebbled with them. They were then quite conspicuous to the naked eye. They appeared both in the interior and at the margin of colonies.

When isolations were made from papillae to tubes of *d*-arabinose broth, the resultant cultures produced a much speedier fermentation than the original stock cultures, at times showing definite acid or acid and gas formation within 24 hours. In contrast to this, transfers made from colonies or portions of colonies free from papillae showed the usual delayed fermentation.

It should be added that as a rule the acid end-products of fermentation of *d*-arabinose are not as evident on agar plates as in broth tubes. Usually the plates remained purple (neutral or alkaline) throughout the entire period of incubation even in the presence of thousands of papillae or daughter colonies. The change in color of the indicator was shown only at the papillae themselves. In broth cultures, as already cited, the entire tube became acid.

FERMENTATIVE BEHAVIOR OF VARIANTS

A number of the quick-fermenting cultures were used for further tests. It seemed of interest to determine whether such cultures, as a result of the "training" in prompt fermentation of the *d*-form of the sugar, might have become altered in their deportment toward the *l*-form or toward other sugars. Accordingly the fermentative abilities of the newly-developed strains were tested with some of the commoner sugars, including several which are not ordinarily utilized. *l*-Arabinose, xylose, rhamnose, glucose, lactose and sucrose were employed for this purpose.

In no instance was any alteration observed. All cultures fermented both the *d*- and *l*-forms of arabinose promptly and there was no change in the deportment toward other sugars. It is perhaps worthy of special note that the slow fermentation of lactose and sucrose characteristic of Sonne dysentery cultures occurred in the usual manner, even though these strains had acquired the ability to ferment *d*-arabinose readily. Evidently the enzymic equipment necessary for the prompt splitting of *d*-arabinose is added to the armament already possessed by the cells without any alteration which can be detected by the usual fermentation tests.

A number of strains of the rapid-fermenters were held for a time to determine whether this newly acquired property would be retained by the cultures for any appreciable length of time. Altogether about 30 cultures were held for periods varying from 10 months to 2 years. After their original isolation from *d*-arabinose serial broth tubes and *d*-arabinose agar plates, they were kept on nutrient agar slants without sugar and transferred about once a month. In the intervals between transfers they were stored in an ice box.

Most of the cultures retained to a surprising degree the ability to ferment the *d*-sugar. Even after a 2-year sojourn on nutrient agar, during which time they had gone through a considerable number of transplants and had not been in contact with the sugar, many of the cultures produced prompt fermentation of the *d*-arabinose. In the majority of cases fermentation was quite distinct in less than 24 hours. When it did not appear or was not pronounced at this time, it usually became evident within 48 hours.

WILL FILTRATES OF ACTIVELY FERMENTING STRAINS ACCELERATE THE FERMENTATION?

It was desired to see whether filtrates of rapidly-fermenting strains would hasten the slow fermentation when added to *d*-arabinose broth. Cultures which produced fermentation promptly were grown in *d*-arabinose broth for periods varying from 16 to 30 hours, at which time they were neutralized with sodium hydroxide solution and passed through Seitz filters. The filtrates were

added in amounts of 0.1, 0.5 and 2.0 cc. to sterile *d*-arabinose broth fermentation tubes. These were incubated 48 hours to test for sterility and then inoculated with stock cultures which gave the usual delayed fermentation. Filtrates of six rapidly fermenting cultures were prepared. Each filtrate was added to a sufficient number of broth tubes so that a series of tests could be made with several additional species as well as with the homologous stock strain.

There was no evidence of any acceleration in the fermentation caused by addition of the filtrates. The interval required for splitting of the sugar was the same as that of control tubes without the filtrate. Thus the presence of a soluble or extracellular "activator" capable of passing through filters was not demonstrated.

SEPARATION OF CELLS AND LIQUID PORTION OF *d*-ARABINOSE BROTH CULTURES AND SUBSEQUENT FERMENTATION

An attempt was made to gain an insight into the course of events by separation of the cells and liquid portion of cultures before fermentation appeared. Cultures were inoculated into 1-per-cent *d*-arabinose broth and incubated for an interval slightly shorter than that required for fermentation. The cultures were then centrifuged to throw down the cells and the liquid portion of the culture was drawn off. In most cases this procedure was applied to cultures about 24 hours before the first evidence of delayed fermentation was expected. That is, if for a certain organism five days were usually required for fermentation, then at the fourth day the culture was centrifuged and the cells separated from the liquid. Just before centrifugation 5 cc. of the culture was removed to a sterile tube, incubated, and the additional time required for fermentation of this part of the original culture was noted. This served to check previous data on the interval necessary for fermentation.

Cells which had been thrown down by centrifugation were immediately taken up and transferred to new tubes of sterile *d*-arabinose broth. In doing this, the volume of new culture to which the cells were added was always the same as that of the

old culture from which the cells had been removed. It will be seen that by this procedure cells which had been in contact with *d*-arabinose in broth for several days were transferred *en masse* and brought into contact with a fresh supply of the sugar before fermentation appeared in the first culture.

In addition to the foregoing procedure, the liquid portion of the culture which remained after centrifugation was immediately put through Seitz filters to remove any remaining organisms and then distributed into sterile test tubes. These tubes of filtrate were then inoculated with the homologous culture, but the cells used for this inoculation were taken from a 24-hour nutrient agar slant of the stock culture and had not previously been in contact with *d*-arabinose. Thus new cells were brought into contact with the sugar after it had been exposed to the effect of the culture for an interval just short of that required for fermentation.

From the foregoing procedures it was hoped to determine whether the cells, during the interval required for delayed fermentation, were changed or adjusted to the sugar (probably in enzymic equipment) in such a way as to enable them to make ready use of the *d*-form of the molecule, or whether instead of such an effect, or perhaps in addition to it, the sugar was being gradually prepared or changed by the cells into a more readily fermentable form.

In table 3 are presented the results obtained on transferring the centrifuged cells to new tubes of *d*-arabinose broth. It will be seen that when cells were removed approximately 24 hours before fermentation and were transferred to new tubes of sugar broth, fermentation in the new tubes occurred promptly, usually within 24 hours. In no instance was there a delayed fermentation corresponding to that ordinarily seen. In a few instances cells were removed 48 hours or more before the usual fermentation. The tests with *A. aerogenes* M4, *S. cholerae-suis* 696 and certain of those with *S. schottmülleri* and *S. dysenteriae* Sonne are examples of this. In general, when cells were removed 2 or 3 days before the usual delayed fermentation, a similar interval elapsed before evident fermentation occurred in the new *d*-arabinose tubes.

Controls were included in all of the foregoing tests by removing

TABLE 3

The effect of transferring cells from d-arabinose broth cultures to new tubes of the medium

CULTURE	TIME REQUIRED FOR FERMENTATION OF *d*-ARABINOSE*	CELLS REMOVED FROM:	FERMENTATION AFTER TRANSFER OF CELLS TO NEW TUBES OF *d*-ARABINOSE BROTH
	days		
E. coli 1.	6	*d*-arabinose broth at 5th day	Prompt†
		Plain broth at 5th day	Slow, 7 to 8 days
E. coli 1.	5	*d*-arabinose broth at 4th day	Prompt
		Plain broth at 4th day	Slow, 5 to 6 days
A. aerogenes 2.	6	*d*-arabinose broth at 5th day	Prompt
		Plain broth at 5th day	Slow, 3 days
A. aerogenes M4.	7	*d*-arabinose broth at 5th day	In 2 days
		Plain broth at 5th day	Slow, 4 to 5 days
K. friedländeri	3	*d*-arabinose broth at 2nd day	Prompt
		Plain broth at 2nd day	Slow, 2 days
S. schottmülleri 822. . . .	5	*d*-arabinose broth at 4th day	Prompt
		Plain broth at 4th day	Slow, 4 to 5 days
S. schottmülleri 822. . . .	5	*d*-arabinose broth at 3rd day	In 2 days
		Plain broth at 3rd day	Slow, 4 to 5 days
S. schottmülleri 822. . . .	6 to 7	*d*-arabinose broth at 3rd day	Slow, 3 days
		Plain broth at 3rd day	Slow, 6 days
S. enteritidis 53.	4	*d*-arabinose broth at 3rd day	Prompt
		Plain broth at 3rd day	Slow, 3 to 4 days
S. dysenteriae, Sonne. .	7 to 8	*d*-arabinose broth at 6th day	Prompt
		Plain broth at 6th day	Slow, 6 to 7 days

* Determined by separate tests in ordinary fermentation tubes and also by removal of a part of the culture (5 cc.) from large tube just before centrifugation. This 5 cc. was transferred to a sterile test tube, incubated, and the time required for fermentation was noted.

† Prompt fermentation implies distinct acid and gas (or acid only in the case of the Sonne dysentery cultures) within approximately the first day after transfer of the cells. In some experiments this occurred within 16 hours while in others 26 to 30 hours were required.

TABLE 3—*Concluded*

CULTURE	TIME REQUIRED FOR FERMENTATION OF *d*-ARABINOSE*	CELLS REMOVED FROM:	FERMENTATION AFTER TRANSFER OF CELLS TO NEW TUBES OF *d*-ARABINOSE BROTH
	days		
S. dysenteriae, Sonne..	7	*d*-arabinose broth at 5th day	In 2 days
		Plain broth at 5th day	Slow, 5 to 6 days
S. cholerae-suis 667....	7	*d*-arabinose broth at 6th day	Prompt
		Plain broth at 6th day	Slow, 4 to 5 days
S. cholerae-suis 696....	6	*d*-arabinose broth at 3rd day	Slow, 2 to 3 days
		Plain broth at 3rd day	Slow, 5 days

cells from cultures of plain nutrient broth at the same time and in the same manner as from the sugar broth. The cells were then transferred to tubes of *d*-arabinose broth. In every case these cells, which had not previously been in contact with the sugar, produced a delayed fermentation. The actual interval required for this effect was at times a little shorter than that normally seen, but it will be recalled that the inoculations were made by transferring cells *en masse* and not by the usual method of carrying over a small inoculum.

One possibility occurred to us which might detract somewhat from the experiments shown in table 3. In the centrifugation and subsequent transfer of cells from older arabinose cultures to new sugar broth tubes, some of the *d*-arabinose from the first tube might have been adsorbed to the cells and thus be carried over to the new tube. While it is unlikely that sufficient sugar would be carried over to account for the vigorous fermentation which was observed in each experiment, nevertheless it seemed best to investigate this point further.

Accordingly several experiments were performed in which the cells, after centrifugation and removal from the first tube of *d*-arabinose broth, were divided into two lots. One lot of cells was added to a proportionate amount of plain nutrient broth without sugar, the other lot was added to new tubes of *d*-arabinose

broth as usual. If sufficient sugar were adsorbed to the cells to account for the prompt fermentation shown in the last column of table 3, then the tubes of plain nutrient broth should become acid.

In no instance did this occur. The nutrient broth tubes which received the cells exhibited pH values of 6.6 to 7.4 at the time (usually 24 hours) when the new *d*-arabinose tubes were distinctly acid, pH 5.5 or less. It was concluded, therefore, that the prompt fermentation obtained upon transferring cells from an older culture was undoubtedly due to actual splitting of the

TABLE 4

The effect of inoculating d-arabinose broth culture filtrates with new cells

CULTURE	TIME REQUIRED FOR FERMENTATION OF *d*-ARABINOSE	*d*-ARABINOSE BROTH CULTURE FILTERED AT:	FERMENTATION AFTER REINOCULATING FILTRATE WITH SAME STRAIN*
	days		
E. coli 1	6	5th day	Slow, 6 to 8 days†
E. coli 5	3	2nd day	Slow, 2+ days
A. aerogenes 2	6	5th day	Slow, 4 to 6 days
A. aerogenes M4	7	5th day	Slow, 11 days
S. schottmülleri 822	5	4th day	Slow, 6 to 10 days
S. enteritidis 53	4	3rd day	Slow, 2 to 3 days
S. dysenteriae, Sonne	7	5th day	Negative throughout

* The cells used for inoculation of the filtrate were taken from 24-hour agar slants prepared from stock cultures. They had not been in contact with *d*-arabinose previously.

† Several tubes were prepared from each lot of filtrate. There was often some difference between these tubes in the time at which fermentation first appeared. This is shown in the table.

newly supplied sugar and not to sugar adsorbed on the cells and carried over from the first culture.

In table 4 experiments are presented wherein the liquid portion of *d*-arabinose broth cultures, after filtration, was reinoculated with cells from agar stock cultures which had not been in contact with the sugar. If any change in the sugar molecule which might facilitate its ultimate breakdown had been brought about as a result of contact with the cells (for an interval just short of that needed for fermentation), then the sugar should be fermented readily by cells from ordinary agar cultures of the same organism.

In no case, however, did this occur. In each instance the fermentation was delayed and the interval was more comparable to that following the ordinary method of inoculation and growth in the sugar broth.

These experiments point to the conclusion that the principal change taking place in the interval before fermentation is an alteration in the fermenting capacity of the cells themselves. When cells are removed after a period of contact with the sugar and are introduced into a new supply of the same, fermentation of this second lot of sugar occurs quite promptly. The process of fermentation by these cells is not appreciably interrupted or delayed by the change in supply of sugar. However, if the cells are taken in a similar way from plain broth (table 3) the usual slow fermentation occurs after placing the cells in sugar broth. Also, the *d*-sugar which has been in contact with cells is apparently not changed in any way which facilitates fermentation, for when the liquid portion of cell-free cultures is inoculated with cells which have not previously been in contact with the sugar, the usual delayed fermentation results.

SUMMARY

A study was made of the delayed fermentation of *d*-arabinose by certain members of the coli-aerogenes, paratyphoid and dysentery groups of bacteria. Increased concentrations of *d*-arabinose in broth, 2.0 per cent and 5.0 per cent, did not accelerate the fermentative process, except in the case of *Aerobacter aerogenes*. The use of shallow layers of medium to afford greater aeration gave no evidence of speedier splitting of the sugar. Anaerobic or partial anaerobic conditions did not appreciably hasten the slow fermentation. In a synthetic medium with *d*-arabinose as the only source of energy most of the organisms were unable to initiate development.

The late fermentation was accelerated by serial passages in *d*-arabinose broth and cultures which had been subjected to this procedure produced fermentation usually within 24 hours. After acquiring ability to ferment rapidly the *d*-form of the sugar, no alteration in behavior toward the *l*-form or toward other common

sugars could be detected. Also, this ability was retained by many cultures for periods up to two years, beyond which the tests were not carried.

On *d*-arabinose agar plates daughter colonies or papillae containing rapidly-fermenting variants gradually appeared within the original non-fermenting colonies.

When cells were removed from *d*-arabinose broth cultures just before fermentation became evident and were transferred to new tubes of the same medium, fermentation appeared promptly. When filtrates of *d*-arabinose broth cultures, from which the cells had been removed just prior to evident fermentation, were reinoculated with new cells which had not previously been in contact with *d*-arabinose, the fermentation was delayed. During the period before evident fermentation the principal change appears to be an alteration in the cells and not a conversion of the *d*-form of the sugar to some more readily assimilable form before it is finally broken down.

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