339. *Multiple Adaptations of* Bact. lactis aerogenes (Aerobacter aerogenes) *to Various Combinations of Substrate.*

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Multiple adaptations of the cells of *Bact. lactis aerogenes* to various combinations of the following have been studied: glucose, glycerol, lactose, succinate, D-arabinose, proflavine, sulphanilamide.

Complete adaptation to more than one is possible, but more commonly there is a compromise with partial adaptation to the individual substances. Sometimes there is incompatibility, and in the case of proflavine and succinate there appears to be a relation between the two kinds of training. In general the adaptation is metastable in that it persists for long periods after removal of the cells from the substrate provoking it, but tends to be removed by appropriate disturbance of the cell economy.

The results are discussed in relation to the two theories of mutation and selection on the one hand and of growth-linked enzymic changes on the other. The second is to be preferred.

According to the chemical theory of the adaptive processes by which bacterial cells become resistant to drugs or utilise new substrates with increased efficiency, the adaptation is essentially reversible, but may show in appropriate circumstances a very considerable degree of metastability (*J.*, 1952, 745).

On transfer to a new medium, increase in growth rate and decrease in lag (the induction period preceding cell multiplication) may occur on continued subculture, and on return to the original medium this so-called training may or may not be lost—as shown by further tests in the second medium. A variety of cases are considered in the paper referred to, and the object of the present study is to illustrate the kinds of behaviour found by experiment on multiple adaptations.

The methods of experiment were as described previously. By training is meant serial subculture of cells in a given medium, the extent of the process being measured by the mean generation time (time taken for number of cells to double in logarithmic growth), and by the lag (intercept of logarithmic growth curve on time axis).

Previous observations in this laboratory made in this way have shown that *Bact. lactis aerogenes* may be trained to show optimum growth rate in maltose, sucrose, and lactose (parallel tests in the three sugars), that this state is compatible with optimum behaviour in glucose (Postgate and Hinshelwood, *Trans. Faraday Soc.*, 1946, **42**, 45), that optimum growth rate in glycerol may be retained during many intervening passages through glucose (Cooke and Hinshelwood, *ibid.*, 1947, **43**, 733), and that, in general, the simultaneous holding of different kinds of training is possible. The theory suggests that, in general, the relations should be rather more complex, and that there should, in some cases at least, be a certain measure of competition between the various forms of adaptation.

The organism used for the present experiments was a strain of *Bact. lactis aerogenes* which has been maintained for some years in the laboratory. The growth media used consisted of phosphate buffer, ammonium sulphate, magnesium sulphate, and the carbon source to which the cells were to be adapted. Where necessary, appropriate amounts of antibacterial drugs were added. When adaptations to more than two substrates were investigated, the bacteria were subcultured successively in the relevant media and then tested simultaneously in them all. Under the standard condition employed a subculture represents about 8—10 successive cell divisions.

In the tables of results the following abbreviations are employed:

G	glucose	Su	succinate
Gy	glycerol	A	D-arabinose
L	lactose	B	broth

Pf indicates a glucose medium containing 2 : 8-diaminoacridine (proflavine) and Sa a similar medium containing sulphanilamide. The history of a strain is indicated as in the following example : A54.Su6.Gy4 means that there had been 54 subcultures in D-arabinose, followed by 6 in succinate and then by 4 in glycerol.

The concentrations of the carbon sources (which are of little influence on lag or growth rate) were from 5 to 25 g./l. as was convenient.

Arabinose–Lactose.—Three strains were investigated, namely A5, A23, and A46, and the behaviour on training to lactose was found to be a function of the number of preliminary subcultures in arabinose. Typical results are shown in Table 1. The symbol ∞ indicates the limiting condition reached after prolonged training.

TABLE 1.

	Strain	M.g.t. (min.)	Lag (min.)
(*a*)	A1	>200	>1500
	A_∞	58	0
	L1	65	100—200
	L_∞	35	0

		M.g.t. (min.) in		Lag (min.) in	
	Strain	A	L	A	L
(*b*)	A5.L8	—	46	100	0
	A5.L35	156	35	580	0
	A5.L45	129	35	850	0
(*c*)	A23.L1	51	48	0	30
	A23.L36	198	34	460	0
	A23.L71	208	38	770	0
(*d*)	A46.L1	63	56	0	0
	A46.L11	75	40	0	0
	A46.L53	68	35	500	0
	A46.L80	77	35	350	0

Comparison of (*b*), (*c*), and (*d*) shows that the arabinose adaptation is more tenaciously held on passage through lactose the longer it has originally been impressed, that a high degree of training to both sugars can be held simultaneously (A46.L11), but that there is competition even in (*d*), where eventually the lag in arabinose shows quite a marked increase. On the other hand, comparison with (*a*) shows that reversion to the untrained state does not come about even in 80 subcultures.

The results of training in the reverse order are shown in Table 2.

TABLE 2.

	M.g.t. (min.) in		Lag (min.) in	
Strain	A	L	A	L
L40	—	35	—	0
L40.A1	250	42	450	0
L40.A3	75	47	—	—
L40.A12	74	45	0	80
L40.A59	49	47	0	120

Comparison with Table 1(*a*) shows that, although even after prolonged passage through the second medium the adaptation to the first is considerably above that of an untrained strain, nevertheless a certain degree of competition is evident. When fully adapted to arabinose the strain has lost some of its adaptation to lactose.

Succinate–Glycerol.—The compatibility of the two kinds of adaptation is here partial. The actual behaviour in the succinate medium shows quite considerable fluctuations, the growth rate being a function of the immediate past history (probably of the precise age of the parent culture). Typical results are shown in Table 3.

The succinate-trained strain does not take the glycerol training completely : nor does it retain quite the full amount of its adaptation to the succinate on passage through glycerol. These facts are revealed by the values of the m.g.t., the lags showing only unimportant fluctuations. (It should be noted that lag is a function of the age of the inoculum, and

that although this is controlled minor fluctuations of an hour or so are of little significance. Real loss of adaptation would be reflected in a lag of at least four hours.)

TABLE 3.

	Strain	M.g.t. (min.)	Lag (min.)
(*a*)	Su1	120—230	500
	Su_∞	35	0
	Gy1	Variable	250
	Gy_∞	35	0

		M.g.t. (min.) in		Lag (min.) in	
	Strain	Su	Gy	Su	Gy
(*b*)	Su6.Gy1	82	39	0	240
	Su6.Gy10	64	49	140	0
	Su6.Gy43	61	41	0	50
	Su6.Gy57	56	45	0	110

Succinate–Arabinose.—A strain which had been trained in succinate for a considerable, but not recorded, number of subcultures was subcultured into arabinose and eventually became moderately well adapted to both substrates. There were some signs of a competition between the two types of adaptation (Table 4). The variations in lag, although appreciable and indicative of impaired adaptation, have to be compared with the value of *ca.* 3000 min. for the untrained strain.

TABLE 4.

	Strain	M.g.t. (min.)	Lag (min.)
(*a*)	Su1	120—230	500
	Su_∞	35	0
	A1	300	*ca.* 3000
	A_∞	58	0

		M.g.t. (min.) in		Lag (min.) in	
	Strain	Su	A	Su	A
(*b*)	Su *x*.A1	60	300	—	530
	Su *x*.A7	44	145	0	530
	Su *x*.A25	83	68	0	60
	Su *x*.A34	66	57	0	400

Arabinose–Lactose–Succinate.—A strain A46.L84 which had been stored in broth for some time without any noticeable change in the state of adaptation was given further passages through arabinose and lactose, and at the end of this process showed a high degree of adaptation to both, as shown in Table 5(*a*). It was then subcultured in succinate, during which process a very considerable impairment of the adaptation to the first two sugars was observed (Table 5*b*).

TABLE 5.

		M.g.t. (min.)			Lag (min.)		
	Strain	A	L	Su	A	L	Su
(*a*)	A1	200	—	—	1500	—	—
	L1	—	65	—		100—200	
	Su1	—	—	120—230	—	—	500
	A_∞	58	—	—	0	—	—
	L_∞	—	35	—	—	0	—
	Su_∞	—	—	35	—	—	0
	A46.L84.B1.A10.L9	66	36	—	0	25	—
(*b*)	A46.L84.B1.A10.L6.Su6	350	200	37	500	250	60
	A46.L84.B1.A10.L6.Su17	350	—	—	700	700	0

The disorganisation of the arabinose–lactose training by the subsequent training to succinate is the most marked competitive effect so far encountered in this series of tests.

Arabinose–Lactose–Succinate–Glycerol.—Several arabinose–lactose trained strains were subcultured successively in succinate and in glycerol. The adaptation to arabinose and

lactose was impaired by this process to varying extents: the glycerol adaptation was imperfect and the succinate adaptation, although not complete, was nearly so. The results are summarised in Table 6.

TABLE 6.

	M.g.t. (min.)				Lag (min.)			
Strain	A	L	Su	Gy	A	L	Su	Gy
A5.L66.Su6.Gy6	55	34	50	75	1400	130	160	270
A23.L42.Su6.Gy6	128	34	54	41	1400	120	130	90
A46.L27.Su6.Gy6	119	46	48	49	800	80	140	25

Proflavine–Succinate.—The strain was trained to grow without lag at a concentration of 100 mg. of proflavine per l. (glucose medium) and was then passed through succinate. From the beginning it showed a considerable degree of adaptation, the m.g.t. being 36 min. and the lag 70 min. only. After 22 passages through succinate the m.g.t. in the medium was 40 min., and the lag in the proflavine–glucose medium was still zero, although the m.g.t. had risen from 45 to 66 min. These results indicate not only a compatibility of the two types of training, but even a certain degree of inter-relationship.

Proflavine–Glycerol.—The proflavine-trained strain was transferred to a glycerol medium, in which adaptation proved to be definitely more difficult than that of the normal strain. After 20 passages the m.g.t. was still 50 min., compared with the value 35 min. reached in normal training by transfer from glucose. These subcultures in glycerol increased the m.g.t. in presence of proflavine from 45 to 68 min. The lag, however, which for the untrained strain would have been over 7 days at 100 mg. of proflavine per l. was still no more than 110 min.

Proflavine–Arabinose.—The trained strain showed considerable resistance to adaptation to D-arabinose, the m.g.t. after 12 subcultures still being as high as 140 min., compared with 76 in a control experiment with an untrained strain also passed 12 times through the arabinose.

Proflavine–Lactose.—After about 8 subcultures the proflavine-trained strain became more or less fully adapted to lactose. After 12 passages in the lactose it had increased its m.g.t. in the drug medium from 45 to 97 min. The lag, however, remained nearly zero.

Sulphanilamide–Glycerol.—Complete adaptation to both drug and carbon source was reached by training first to 1000 mg. of sulphanilamide per l., and then subjecting the strain to 20 passages in glycerol. The final m.g.t. in glycerol was 35 min., and that in the glucose medium with drug 36 min. The lags both approximated to zero. The rate of adaptation of the drug-trained strain to the glycerol was rather slower than normal.

Sulphanilamide–Succinate.—The results were generally similar to those found with glycerol.

Sulphanilamide–Arabinose.—Complete training to the arabinose was attained after about 20 subcultures (m.g.t. 58 min., lag zero). The rate of training appeared to be lower than normal.

Sulphanilamide–Lactose.—The strain trained to 1000 mg. of sulphanilamide per l. showed considerable resistance to training to lactose. There was a long lag on the first transfer, and this persisted through several subcultures. Eventually after 50 subcultures the lag was zero and the m.g.t. 35 min.

Proflavine–Sulphanilamide–Succinate.—The strain trained to 100 mg. of proflavine per l. was subcultured successively in sulphanilamide media containing respectively 250, 509, and 1000 mg./l. and then passed further through the last of these. It was then trained to succinate in parallel with a culture transferred directly from the usual glucose medium. These two strains showed parallel behaviour except that the drug-trained bacteria had already acquired some degree of adaptation to the succinate.

Proflavine–Sulphanilamide to Arabinose, Glycerol, or Lactose.—In all cases the strain trained to the two drugs offered some resistance to further training, especially with lactose, but the final state was not investigated.

Proflavine–Arabinose–Succinate.—A strain was fully trained to 100 mg. of proflavine

per l. and then subcultured 14 times in D-arabinose and 14 times in succinate. It then proved to be fully trained to none of these, but to show a better performance in all media than the untrained strain would have shown, that is, a compromise seemed to have been reached. The lag in succinate was zero, but the m.g.t. was ten minutes higher than that of the fully trained strain. In arabinose the m.g.t. was 205 min., and the lag 750 min. (compared with zero for the fully trained strain and over 1500 min. for the normal strain). In proflavine at 100 mg./l. the m.g.t. was 64 and the lag 1000 min. (optimum value zero, original value >7 days).

Proflavine–Arabinose–Lactose.—The proflavine-trained strain (as above) was given 14 passages in arabinose and 14 in lactose. As a result the strain was nearly fully adapted to lactose (lag 70 min. and m.g.t. 42 min. compared with 35 for complete adaptation). The lag in arabinose was 1000 min. and the m.g.t. 66 min. (optimum 58 min. and original >200 min.). The lag in proflavine had risen to more than 24 hours.

Once again, competition and compromise are apparent.

Proflavine–Sulphanilamide–Succinate–Glycerol.—The proflavine-trained strain was passed through sulphanilamide four times (the concentration being increased to 1000 mg./l.) and then given 6 passages in succinate and 6 in glycerol. The lag in proflavine remained quite short (300 min.) but the m.g.t. increased to 77 min. The succinate training was complete, but in glycerol the m.g.t. was 163 min. compared with the optimum of 35 min. Tested in sulphanilamide at 500 mg./l. the strain showed zero lag, but an m.g.t. of 78 min.

Proflavine–Sulphanilamide–Succinate–Glycerol–Lactose.—The strain finally tested was Pf*x*.Sa4.Su6.Gy5.L8, *x* being a very large number. It showed a lag of 1150 min. in proflavine at 100 mg./l. (cf. >7 days and zero for the extremes): in sulphanilamide at 500 mg./l. the lag was 200 min. The results are summarised in Table 7.

TABLE 7.

	M.g.t. (min.) in				
	Pf	Sa	Su	Gy	L
Multiply-trained strain	85	45	58	42	200
Optimum	45	33	35	35	33
	Lag (min.) in				
	Pf	Sa	Su	Gy	L
Multiply-trained strain	1150	200	0	250	600
Optimum	0	0	0	0	0
Initial value	>7 days	—	500	250	200

The lactose-adaptation has been very adversely affected. In the other cases there is some sort of a compromise.

Transfers between Glucose Media with and without Proflavine.—After adaptation to proflavine the growth rate in glucose of the normal strain is found to be lowered. Judging from the changes brought about by acriflavine in yeast cells one may infer that some of the oxidative mechanisms of the cell have been damaged (Slonimski and Ephrussi, *Ann. Inst. Pasteur*, 1949, **77**, 47). Serial subculture in the original drug-free glucose medium restores the normal growth rate, and it now becomes a matter of interest to know how the proflavine adaptation fares during this "re-training" to normal behaviour in glucose.

The normal strain of *Bact. lactis aerogenes* was transferred from the usual glucose medium to one containing 20 mg. proflavine per l. and then successively to media containing increasing amounts up to 90 mg./l. At intervals during the training process the growth characteristics were measured, both in the medium containing the highest concentration of proflavine to which the cells had so far been exposed, and in the drug-free medium. Various trained strains were then tested at intervals during serial passage through the glucose medium, and when they were found to have returned almost to normal were re-tested in presence of proflavine for retention or loss of adaptation. Sugar tests were also made at intervals (according to the procedure laid down in Reports on Public Health and Medical Subjects, No. 71, The Bacteriological Examination of Water Supplies, H.M.S.O., 1939).

The results of these experiments are summarised in Table 8. For small degrees of proflavine-adaptation the growth rate in the glucose medium is not noticeably impaired. But as training is carried further the trained strain shows signs of damage, the m.g.t. in the drug-free medium rising to over 40 min. Serial passages bring this back to normal. At the end of the re-training process the proflavine-adaptation itself shows signs of appreciable regression, but is still much greater than it was for the original untrained strain, lags of 3—6 hours having developed in place of zero for the fully trained cells but in place of more than 24 hours for the original.

TABLE 8.

History of training: subcultures in Pf medium at mg./l.				M.g.t. in Pf at max. conc. in training (min.)	M.g.t. in glucose medium after number of "re-training" subcultures shown in parentheses (min.)	Test of "re-trained" strain in Pf: m.g.t. (min.)	lag (hr.)
20	40	60	70	—	—	—	—
1				—	(2)29, (4)32	—	—
3				—	(2)29	—	—
3	2			—	(3)32	—	—
3	3			55	(2)46, (3)32, (4)42, (10)35	—	—
3	3	3		49	(3)45, (10)40, (12)40, (17)32	—	10
3	3	6		—	(4)41, (9)41, (14)39, (16)31	50	5·5
3	3	6	1	—	(3)48, (8)47, (13)52, (15)37, (17)34	50	6
3	3	6	4	—	(4)47, (6)49, (9)42, (11)36, (13)38	51	5

Notes: The subcultures recorded under history of training are additive. The lags in the last column would all have been well over 24 hours for untrained strains.

In these experiments the training was carried up to 90 mg./l. The m.g.t. in presence of proflavine was then 56 min. and the lag zero. The initial value of the m.g.t. found on testing in the drug-free glucose medium was, in different tests, 36, 44, and 37 min. and in 8 or 9 subcultures in glucose it had not returned fully to normal.

The strain after 8 passages at 70 mg./l. and one at 80 mg./l. showed an m.g.t. on return to the drug-free medium, which fell from 47 to 33 at the 6th passage and rose to 36 min. at the 8th. The lag in the proflavine medium was then 3½ hr.

The sugar tests of the proflavine-trained strain were in some respects abnormal, notably in failure to give acid and gas with sucrose. The Voges–Proskauer test was also negative. Subsequent passage through drug-free glucose medium restored these tests to normality.

DISCUSSION

One general conclusion that can be reached is that with this organism nearly complete training to two or more substrates can occur, but that more usually there is a compromise. The first type of case seems to be characteristic of substances which are metabolised by similar reaction routes, for example, glucose and glycerol, arabinose and lactose. The second type probably indicates a competition between metabolic routes: it is exemplified by the system succinate–lactose, where the incompatibility is rather marked.

Evidence exists for the presence of alternative routes of this kind. In the re-training in glucose of the proflavine-damaged strains composite growth curves showing two phases, one of longer, the other of shorter m.g.t., were sometimes observed. This happens when growth by one route with a shorter lag is superseded by more rapid growth associated with a longer lag (cf. Dean and Hinshelwood, *J.*, 1951, 1157).

Training to drugs is compatible with adaptation to carbon substrates, but various specific influences are in evidence. The example of proflavine is interesting. As has been pointed out, this substance causes damage to the oxidative mechanisms of the cell, according to Slonimiski and Ephrussi (*loc. cit.*) to the cytochrome system. According to Keilin and Hartree (*Proc. Roy. Soc.*, 1940, *B*, **129**, 227) the succinic dehydrogenase system is closely related to cytochrome. Prolonged training to proflavine may, therefore, lead to an increase in the amount of this system to compensate for its decreased activity. On transfer of the trained cells to succinate, therefore, they may well prove to be much more fully adapted than normal cells, as indeed is found.

Another important characteristic of the general picture presented by these results is that the state of the trained bacterial cell is more often metastable than stable, and, although

the adaptation may show a high degree of persistence, it does suffer slow changes on very prolonged subculture. The stability is in a large measure a function of the adaptive history, as illustrated by the results for arabinose and lactose.

About the general interpretation of adaptive phenomena, there are, of course, two schools of thought. According to one, training depends upon chance mutation of genes followed by the selection of those mutants which are favoured by the particular environment. According to the other, modification of the enzymic make-up of the cell occurs in response to changed reaction rates, in the manner outlined in a previous paper (*J.*, 1952, 745).

In its application to the present results the mutation-selection theory demands several special assumptions which detract from its probability. The process of training is often slow, gradual, and continuous. There is no rapid emergence of a fully adapted mutant strain as there would be if the change were controlled by a single major mutation. It is necessary to postulate a system of polygenes, so that at any stage the differences between individuals are small. Loss of training has to be attributed to reverse mutations, and where a compromise between two types of adaptation is reached this has to be explained by a selection of polygenes after the appropriate and rather elaborate series of mutations and reverse mutations. To account for the dependence of persistence on previous history it may also be necessary to postulate special genes controlling, not only the character, but also the stability of the training.

Such assumptions are not impossible ones, but they are not based upon any very probable physico-chemical grounds. On the other hand, the hypothesis of changed enzyme constitution, although not without its own complications, is more satisfactory from a chemical point of view. It is, indeed, itself a selection theory in one sense, since the changes in enzyme proportions which are brought about by altered reaction rates themselves constitute an internal selection of one type of matter within all the cells, compared with the selection of a few special cells. Both kinds of effect may in fact occur, but the reference of the adaptation to the bulk of the population is to be preferred, for the examples considered, in view of the fact that in several similar cases there is direct evidence for the response of the majority of the cells (Jackson and Hinshelwood, *Proc. Roy. Soc.*, 1949, *B*, **136**, 562; **137**, 88; Baskett and Hinshelwood, *ibid.*, 1951, *B*, **139**, 58; Kilkenny and Hinshelwood, *ibid.*, p. 73; Baskett, *ibid.*, in the press; Dean and Hinshelwood, *J.*, 1951, 1157). According to the theoretical findings of the earlier paper (*J.*, 1952, 745) there should, on the whole, be some degree of antagonism between different adaptations, unless the enzymes concerned are closely related, or unless an enzyme capable of handling both substrates is present. With glucose–arabinose or glucose–glycerol this may occur. With adaptation to proflavine and succinate there is also the possibility that a common enzyme system expands during the training. But more often apparent compatibility of training is to be explained in terms of metastability and slowness of reversion, and this idea seems in general to be borne out by the experiments. One of the cases dealt with theoretically envisaged an increase in lag in an original substrate caused by intervening passages in a second substrate : there are several indications of such an effect among the experimental results described.

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