The Resistance of Bact. lactis aerogenes (Aerobacter aerogenes) *to* 8-*Hydroxyquinoline.*

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Cultures of *Bact. lactis aerogenes* can be adapted, by growth in media containing 8-hydroxyquinoline in progressively increased concentrations, to yield resistant strains. The resistance is graded to the maximum concentration in previous exposures. The catalase activity of adapted strains appears to be lower than normal.

The response of *Bact. lactis aerogenes* (*Aerobacter aerogenes*) to toxic or antibacterial agents varies, as with other organisms, both in type and in degree. Forms resistant to phenol develop with extreme difficulty if at all, while " training," for example, to sulphonamides, occurs with comparative ease. This means that the study of any new example possesses a certain interest of its own, which is increased by another factor, namely, the relation between the quantitative degree of the response and the concentration of the agent to which the bacteria have been exposed. In their simplest form adaptation theories of drug resistance involve a graded relation between the resistance of the trained strains and the concentration of the drug to which the cells have been subjected, while in its simplest form the theory of the selection of independent resistant mutants would predict the emergence of a few specific resistant types not closely defined by the exposure. Modifications of either theory provide for a more complex picture. Nevertheless the characteristic relation between " training concentration " and response remains of interest in each separate case.

The present work deals with 8-hydroxyquinoline which further possesses one specific point of interest. If this well-known chelating agent acted by blocking essential and irreplaceable trace metals in the cell, no adaptation would presumably be possible, though metallic antagonists could be found. In fact, however, well-marked and continuously graded resistance develops with comparative ease.

Experimental

Experimental Methods.—The organism was that used by Phillips and Hinshelwood (*J.*, 1953, 3679), and the media were the same except that the iron content was more exactly defined, 0·2 mg./l. of added ferrous sulphate being present. Other heavy metals were confined to those present in " AnalaR " reagents, or in water doubly distilled from all-glass apparatus.

The criterion of resistance is the absence of lag in the growth of cells transferred from a dividing culture to a fresh supply of medium containing the 8-hydroxyquinoline. With a culture not previously exposed the lag rises from zero in a drug-free medium towards infinity with about 10 mg./l. as shown in the left-hand curve of the Figure.

The " training " procedure consisted in serial culture in media containing a fixed amount of the 8-hydroxyquinoline until no further improvement took place. The relation between the lag of the resulting strain and the concentration of the drug in a series of test media was then determined. The strain trained to this first stage was then given a further series of culturings at a given greater concentration, and so on. Lag–concentration curves were determined for each stage of training as shown in the Figure.

Results and Discussion

The lag–concentration curve of the original strain is the left-hand curve. The generation times (time required in logarithmic growth for number of cells to double) are given in Table 1. The generation times rise much less steeply than the lags, but this has little

Table 1. *Concentration of* 8-*hydroxyquinoline and generation time.*

Concn. (mg./l.)	0	1	3	4	5	6	8	10
Gen. time (min.)	32	34	35	43	47	41	41	49

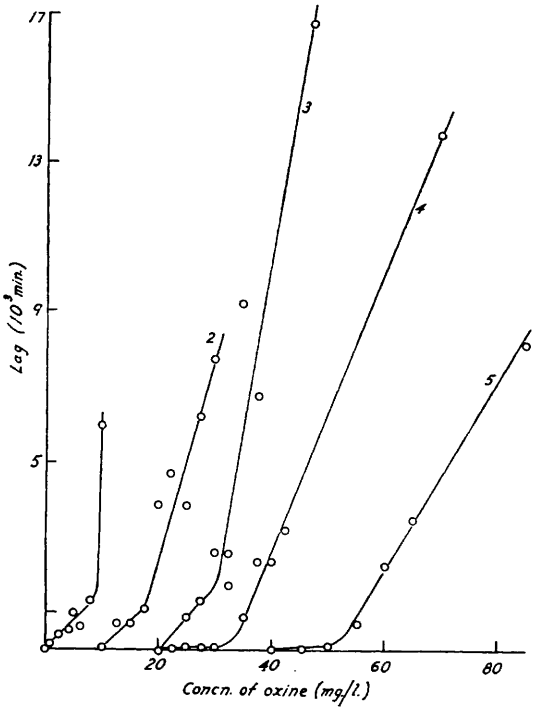
significance : the measurements cannot, from their nature, be made until growth has begun and by this time adaptation (or selection) is already under way.

The resistant strains do not emerge at once (as they would if pre-existent single-step mutants were selected). This is indicated in Table 2. A lag of 50 min. being of little significance, the last strain referred to in Table 2 was used for the training to 20 mg./l. It

TABLE 2.

No. of subcultures at 10 mg./l.	1	2	11
Lag in test at this concn. (min.)	6000	1900	50

was tested after 16 and 31 culturings at this higher concentrations with results shown by the appropriate curves in the Figure. It will be seen that the resistance continues to



Curve 1, Untrained strain.
Curve 2, Strain given 11 subcultures at 10 mg./l.
Curve 3, ,, 16 ,, 20 mg./l.
Curve 4, ,, 31 ,, 20 mg./l.
Curve 5, ,, 16 ,, 40 mg./l.

increase over a considerable number of subcultures (each of which in the conditions of growth involves about 8 successive cell divisions).

The right-hand curve shows the behaviour of the strain which had received 16 subcultures at 40 mg./l.

The curves are spaced out in a way which shows a more or less continuously graded response. No single parameter will adequately express their displacement since they become progressively less steep as they are displaced to the right. The most important characteristic, however, is the point at which the very rapid increase of lag sets in. For an approximate measure of this we may take the concentration at which the lag reaches a standard value of 1000 minutes. On this basis Table 3 may be constructed. There is a distinct parallelism between the numbers in the top row and those in the bottom. The latter increase rather more rapidly. This, combined with the progressive flattening of the curves, suggests a tendency for cells sufficiently trained to reach a state where no concentration of the 8-hydroxyquinoline has any further effect. Reasons for such behaviour have been discussed elsewhere (Peacocke and Hinshelwood, *J.*, 1948, 2290). Nevertheless over

a wide range the resistance developed is closely governed by the maximum concentration to which the cells have been exposed. This behaviour is characteristic either of a physiological adaptation, or of a complex series of mutations each attended with a small increase only in the resistance. It is not proposed to debate here these two alternatives.

TABLE 3. *Family of lag–concentration curves (units are mg./l.).*

Concn. of previous exposures	0	10	20	40
Concn. in test at which lag is 1000 min.	7	17	36	56
Increment caused by training	0	10	29	49

The main interest of the result is that whatever part of the cell mechanism the 8-hydroxyquinoline inactivates, or whatever growth requisite it removes, the interference can be compensated for.

With the idea that the 8-hydroxyquinoline might have a strong action on the iron-containing catalase of the cells, and that there might be significant changes in the readiness of the adapted cells to form this enzyme, experiments were made on its production during the growth cycle of trained and of untrained cells. The method of study was that used by Cole and Hinshelwood (*Trans. Faraday Soc.*, 1946, **43**, 266). Cells which had become adapted to 20 mg. of 8-hydroxyquinoline per l. were found to form less catalase at any stage of the growth cycle, but the formation began earlier and reached its maximum sooner than with the original unadapted strain. No detailed explanation of this is offered at the moment.

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