Tetrathionase: The Differential Effect of Temperature on Growth and Adaptation

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SUMMARY: Cells of a tetrathionate-reducing coliform organism growing in semi-anaerobic conditions gained no advantage from the presence of tetrathionate at 41°, but at lower temperatures (e.g. 34°) they grew much better with than without it. When freely supplied with oxygen, the cells grew about as well at 41° as at 34°. The adaptive formation of tetrathionase in washed suspension has already been shown to diminish with increase of temperature from 34° to 44°, whereas the activity of the enzyme when formed increases with temperature in this range; nitratase, on the other hand, is still actively formed at temperatures as high as 44°. It is clear that whatever factors may be necessary for adaptation, one of them is more sensitive to heat than either nitratase formation or the overall growth process.

In previous work the following facts about the bacterial enzyme system tetrathionase were established:

(1) *Salmonellae* and a number of other Gram-negative bacteria can reduce tetrathionate quantitatively to thiosulphate (Knox, Gell & Pollock, 1943). Both the activity of the fully developed enzyme and its adaptive formation can be demonstrated in washed suspension in the presence of a suitable H donator (Pollock & Knox, 1943; Knox & Pollock, 1944).

(2) The velocity of reduction by adapted cell suspensions increases with temperature at least up to 40° or even higher. Adaptation, on the other hand, is almost completely suppressed at 41–42°, while it occurs rapidly at 37° and even lower temperatures (Pollock, 1945).

(3) The reduction of tetrathionate is of value in growth to those organisms that can reduce it. Tetrathionate is a selective hydrogen acceptor and an alternative to oxygen; it is not so efficient as oxygen but it enables a broth culture to reach in a few hours a population two to three times the population reached in unaerated broth (Knox, 1945).

The relation between adaptation (which may involve the biosynthesis of a specific enzyme or enzyme system) and growth of a bacterial culture (synthesis of bacterial protoplasm as a whole) is one of intriguing interest. A study has now been made of the effect of temperature on the capacity of a bacterial culture to reduce tetrathionate during growth.

METHODS

The culture used was the coliform organism of intermediate type labelled '1433' (Pollock, 1946). Cells from an overnight broth culture prepared from stock agar slopes were heavily inoculated, usually so as to give just visible turbidity at the start, into 60–80% tryptic heart broth containing 0.05M phosphate at pH 7.6. Tetrathionate was used in a concentration of 0.02M. Cultures were incubated in thermostatically controlled water-baths at different temperatures,

static cultures in 6 in. $\times$ $\frac{3}{4}$ in. or 6 in. $\times$ $\frac{5}{8}$ in. tubes, but aerated cultures in larger tubes through which oxygen was bubbled and to which a few drops of undecanol were added to suppress frothing (Linggood & Fenton, 1947). Either the culture tubes themselves or suitable samples, diluted if necessary, were removed for estimation of turbidity in a Hilger Biochem absorptiometer. In some experiments total counts were performed using a Helber counting-chamber and dark-ground illumination, and viable counts by surface plating of suitable dilutions on to nutrient agar.

RESULTS

Fig. 1 illustrates the growth of organism 1433 in buffered broth at 34° and 41° under different conditions. In freely oxygenated broth growth at 41° was at least as good as at 34°. There was some variation in different experiments as it

Fig. 1. Growth of organism 1433 in buffered broth at 34° and 41°. At 34°: A, oxygenated; B, semi-anaerobic; C, tetrathionate (0.02M). At 41°: D, oxygenated; E, semi-anaerobic; F, tetrathionate (0.02M).

was difficult to maintain exactly equivalent oxygenation at the two temperatures. In some experiments growth at 41° was initially a good deal faster than at 34°. In static broth cultures growth was poor at both temperatures as the limiting population was reached after only a few cell divisions, but growth at 41° was slightly less than at 34°. In tetrathionate broth growth at 34° was good, though the cultures never reached the same density as in oxygen, but at 41° it was poor. These experiments showed that although with a free supply of O₂ the cells grew equally well at 41° and at 34°, it was only at the lower

temperature that they gained an advantage from using tetrathionate as an alternative H acceptor to oxygen.

Measurement of tetrathionate reduction by the cells in this experiment at the two temperatures showed that at 34° tetrathionate was rapidly reduced to thiosulphate, whereas at 41° the amount reduced was negligible.

Fig. 2 shows for comparison the behaviour of adapted and unadapted cells of organism 1483 in washed suspension at the two temperatures used in the growth experiments. Reduction of tetrathionate at 41° was, as expected, faster than at 34°, whereas adaptation by unadapted cells was much faster at 34° than at 41°, although not completely suppressed at the higher temperature.

Fig. 2. Reduction of tetrathionate by washed suspensions of organism 1483 at 34° and 41°. A, adapted cells; B, unadapted cells.

It is not certain that adaptation during growth is the same process as adaptation in washed suspension, but it is interesting to observe that the known temperature-sensitivity of the adaptive mechanism in washed suspension is paralleled by the behaviour of the same organism during what may be described as 'adaptive' growth. It seems that adaptation is a necessary preliminary for adequate growth in semi-anaerobic conditions—since adequate growth occurs, even in a rich medium, only at temperatures low enough to leave undamaged the mechanisms of adaptation. There was found to be a fairly wide temperature optimum for 'adaptive' growth, between 32° and 38°. Above this range 'adaptive' growth still occurred at 40° but declined very sharply between 40° and 41°, until at 42° it was completely suppressed.

In view of the known selective value of tetrathionate, a few experiments were performed with mixed cultures of the tetrathionate reducer 1433 and a strain of *Bact. coli* which did not reduce tetrathionate. Tubes of heart broth and of tetrathionate heart broth, containing mixtures of the two organisms in about equal numbers, were incubated at 34° and 41°. The heart-broth tubes were freely oxygenated throughout the experiments. The results, as might be expected, were erratic; but in one experiment whereas the proportion of reducers to non-reducers either remained unchanged or decreased in oxygen at 34° and 41° and in tetrathionate at 41°, it increased in tetrathionate at 34°, showing that the reducer gained an advantage over the non-reducer in the presence of tetrathionate only when growing at a temperature at which adaptation and therefore utilization of the tetrathionate occurred.

The failure of organism 1433 to reduce tetrathionate during growth at 41° was presumably due to a noxious effect of temperature, and perhaps of tetrathionate as well, on some metabolic process in the cells. The least sensitive mechanism is almost certainly not the tetrathionase enzyme system, since in washed suspension this is more active at 41° than at 34°. Nor is it likely that the growth of the whole bacterial cells is much affected, since freely aerated cultures of organism 1433 grow as well at 41° as at 34°, not only in the absence, but also in the presence of tetrathionate. All the evidence suggests that it is the process of adaptation as opposed to the overall growth process that is differentially and selectively interfered with by the increase of temperature.

To what extent this inhibition of adaptation can itself be explained by an increased toxic effect of tetrathionate at higher temperatures is not clear. At this point some experiments with nitratase are relevant.

Cultures of organism 1433 were incubated in the usual way at different temperatures, but with nitrate instead of tetrathionate. At 41°, whereas growth in tetrathionate broth was very poor, in nitrate broth it was as good as at 34°, and nitrate still stimulated growth up to 42–43°. Even the presence of tetrathionate in addition only partially suppressed this stimulatory effect. These experiments suggested that nitratase adaptation is less thermolabile than tetrathionase adaptation. This has been further demonstrated by direct experiments on the effect of temperature on the two adaptive processes in washed suspension. A considerable adaptive production of nitratase occurred even at 44·5°, whereas tetrathionase adaptation was almost abolished at 42° and considerably inhibited at 40° (Pollock & Wainwright, unpublished observations). Elsewhere we have shown (Jebb, Knox & Tomlinson, 1950) that tetrathionate concentrations down to 0·005 M do have some inhibitory effect on adaptation; but since the effect of tetrathionate on 'adaptive' growth in nitrate is not great even at 42° it seems unlikely that tetrathionate should suppress the formation of 'its own' enzyme even more than that of another. Finally, it was found in growth experiments in the presence of tetrathionate at 34° and 42°, that 'adaptive' growth still failed to occur at the higher temperature even when the tubes contained as little as 0·0025 M tetrathionate.

DISCUSSION

If tetrathionate is to give an effective stimulus to the growth of cells which can use it as a hydrogen acceptor when oxygen is limited, it is clear that growth must occur at a temperature at which adaptation can take place, namely, at around 32–37°. At higher temperatures, appreciable growth in a medium in which tetrathionate is the only effective hydrogen acceptor fails because adaptation is suppressed. This suppression may in turn be merely a reflexion of the fact that tetrathionate is a somewhat toxic substance whose toxicity might be expected to increase with rise in temperature. But whatever the explanation, it is evident that here is an example of a specific adaptation process being almost completely suppressed at a temperature at which growth seems quite unimpaired. The reverse might perhaps have been expected, since increase in bacterial protoplasm (growth) is presumably the result of the co-ordinated working of many enzyme systems, and the optimum temperature for growth no doubt depends on a balance resulting from the temperature optima of many of these systems, the most heat-sensitive of which might be expected to be growth-limiting. With tetrathionase it seems that while this enzyme system when formed is less sensitive, the actual process of its formation is much more sensitive to increase in temperature than is the growth process as a whole. There seems to be little information as to the heat-sensitivity of other adaptive enzymes. It is interesting that nitratase adaptation seems to be much less heat-sensitive than tetrathionase; this is further evidence of the comparative independence of these two adaptation processes.

It is only in recent years that the importance of adaptation in bacterial growth has been appreciated, and an investigation into the temperature optima of different adaptive systems may be expected to add considerably to our understanding of the growth processes of the bacterial cell.

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REFERENCES

- JEBB, W. H. H., KNOX, R. & TOMLINSON, A. H. (1950). Some factors influencing the rate of formation of tetrathionase. *J. gen. Microbiol.* **4**, 374.
KNOX, R. (1945). The effect of tetrathionate on bacterial growth. *Brit. J. exp. Path.* **26**, 146.
KNOX, R., GELL, P. G. H. & POLLOCK, M. R. (1943). The selective action of tetrathionate in bacteriological media. *J. Hyg., Camb.*, **43**, 147.
KNOX, R. & POLLOCK, M. R. (1944). Bacterial tetrathionase: Adaptation without demonstrable cell growth. *Biochem. J.* **38**, 299.
LINGGOOD, F. V. & FENTON, E. L. (1947). The production of diphtheria toxin by submerged culture in shaking flasks. *Brit. J. exp. Path.* **28**, 354.
POLLOCK, M. R. (1945). The influence of temperature on the adaptation of 'tetrathionase' in washed suspensions of *Bact. paratyphosum* B. *Brit. J. exp. Path.* **26**, 410.
POLLOCK, M. R. (1946). Adaptation of 'nitratase' in washed suspensions of bacteria. *Brit. J. exp. Path.* **27**, 419.
POLLOCK, M. R. & KNOX, R. (1943). Bacterial reduction of tetrathionate. *Biochem. J.* **37**, 476.

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