SOME EFFECTS OF HEAVY WATER ON THE GROWTH OF *SERRATIA MARCESCENS*

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Summary

When *Serratia marcescens* is grown on solid or liquid media containing heavy in place of light water, pigment production ceases. When the media contain mixtures of heavy and light water, pigment production is inversely proportional to the heavy water concentration. The results of the biochemical tests used in the identification of the organism are the same for the deuterated and normal cells with the exception that acetyl methyl carbinol production cannot be detected. It is suggested that the effects of deuteration on bacteria are of two types, transient ones following the change from one isotopic medium to the other, and permanent ones caused by the "toxicity" of deuterium. This view is compared with that of other authors.

I. Introduction

The overall effect of deuterating biological systems appears to be the depression of metabolic rate (Wiberg 1955). This is a consequence of the fact that the deuterium isotope effect is in the direction of reduced reaction rates. It is not surprising, therefore, that deuteration has been reported to have a depressing action on the growth of plants and animals, and to decrease the rate of photosynthesis and of alcoholic fermentation (Chance and Allen 1946).

More recently some specific effects of deuterium on biological systems have been observed. The most striking of these is the action of deuterium in preventing cell division rather than cell growth. Borek and Rittenberg (1960) have shown this in the case of bacteria where an early result of deuteration which is often seen is the production of giant cells.

It was noticed in this Laboratory that when *Serratia marcescens* is grown on a heavy water medium its normal pigment production ceases. This effect is interesting in that it is a specific effect which persists after adaptation to the new isotopic environment has occurred and presumably persists as long as the organism is in contact with heavy water.

II. Materials and Methods

(*a*) *Test Organism*

A wild strain of *S. marcescens* freshly isolated from the Woronora River, N.S.W., was used as the test organism and when tested in the appropriate light water media was found to have the following biochemical characteristics: no visible gas from glucose; gelatin liquefied; nitrites produced from nitrates; V.P. positive, indole not produced. The biochemical tests and viable counts were carried out by the methods recommended by the Society of American Bacteriologists (1957).

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(*b*) *Analytical Methods*

The method used to examine the pigment produced by the bacterial cells was as follows. An equal volume of A.R. acetone was added to an aliquot of the whole broth culture which was then stored overnight in the dark, at room temperature. The pH was then adjusted, where necessary, to 8·0 and the cells were disrupted with an M.S.E. ultrasonic vibrator equipped with a $\frac{3}{8}$-in. titanium probe operating at 20,000 cycles per second for 30 sec.

Spectrophotometric examination of the sample was carried out with a model DK2 Beckman ratio-recording spectrophotometer. Nutrient broth–acetone was used as a blank. A scan of the blank against water appears in Figure 1. Absorbancy measurements at particular wavelengths were carried out with a Unicam SP600 spectrophotometer. Beer's law was shown to be obeyed below absorbancies of 0·4 and, where necessary, samples were diluted to bring them within this range.

(*c*) *Heavy Water Media*

Difco dehydrated media were used throughout the experiments, being reconstituted either in H_2O or D_2O and sterilized. Intermediate concentrations were obtained, where necessary, by aseptic mixing of the two basic media in appropriate amounts. The D_2O media were sterilized in a pressure-cooker containing D_2O so as to minimize isotopic degradation. In order to estimate the isotopic degradation caused by handling and by the use of dehydrated media containing hydrogen, the distillate from D_2O media was assayed and found never to be more than 4% below the isotopic purity of the original D_2O used. Isotopic purity was determined by measurement of the variation in refractive index (Kirschenbaum 1951).

To ensure that cultures containing heavy water did not become degraded by atmospheric absorption of H_2O the cultures were aerated with filtered air which had been saturated with water of the same isotopic composition as the cultures.

The media used were reconstituted in heavy water which conformed to the following specifications:

Tritium content less than 25 μc/ml,

Isotopic purity 99·7%,

^{18}O enrichment no more than 0·34 atoms % ^{18}O excess,

Conductivity 10 μmho at 20°C.

III. Experimental Results

Sets of six test tubes of nutrient broth in which the H_2O was progressively replaced by D_2O in 20% steps were inoculated with *S. marcescens* and incubated at room temperature (20–22°C) for 96 hr. At the end of this period one set of cultures was examined spectrophotometrically as described. Another set was treated similarly, except that the cells were removed by centrifugation instead of being disrupted by vibration, and the supernatant was scanned. In both cases the graphs were similar but the cultures subjected to ultrasonic destruction showed improved resolution of the peaks and this method was therefore used throughout.

Figure 1 shows the results obtained when a set of cultures prepared as described above was scanned. The organism grown in 100% H_2O shows the normal peaks at 490 and 535 mμ (Wilbein, Green, and Rappoport 1956; Monk 1957) associated with the pigments produced by *S. marcescens*. Descending the scale, the 80% and 60% H_2O cultures show similar curves. However, as the deuterium content increases the organism gradually ceases producing these two pigments and in the 100% D_2O culture there is no sign of either pigment.

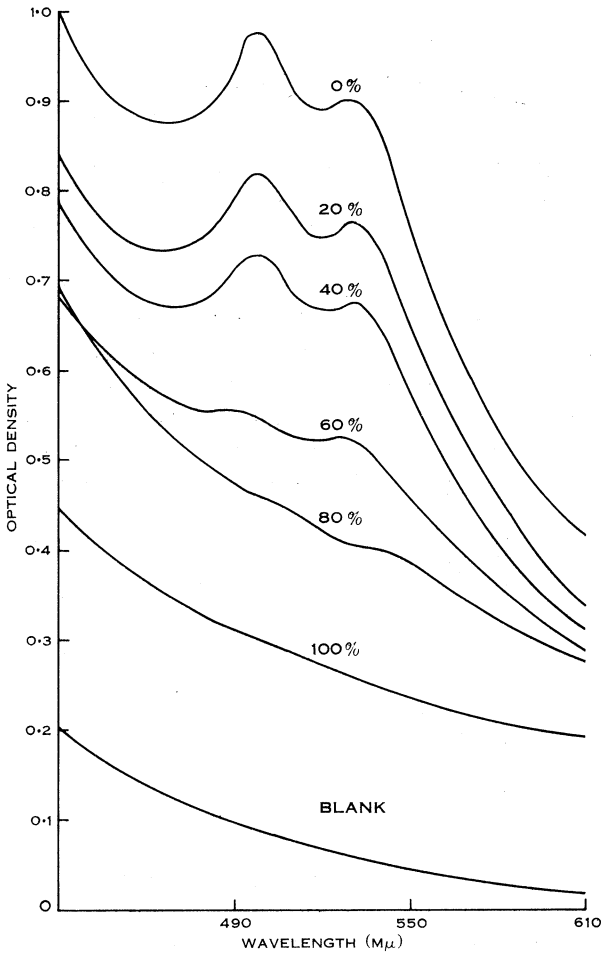

Fig. 1.—Visible spectrum of pigments produced by *Serratia marcescens* growing in D_2O broth. The top six curves represent 0, 20, 40, 60, 80, and 100% D_2O broth, and the lower curve a nutrient broth–acetone blank against water.

Figure 2 gives viable counts and optical density readings at 660 mμ of a series of broth cultures after incubation for 72 hr at room temperature.

Figure 3 shows the results of optical density readings taken at 660, 530, and 485 mμ on cultures treated similarly to the batches which were examined with the recording spectrophotometer. In the range 0–90% D_2O the amount of pigment

produced is inversely proportional to D_2O concentration. The 660 mμ readings, though taken on disrupted cells, would give an indication of the total cell mass. Whereas cell mass falls approximately 0·75 of a unit between 0 and 90% the total pigment produced falls about 2·25 units—a ratio of 3 : 1.

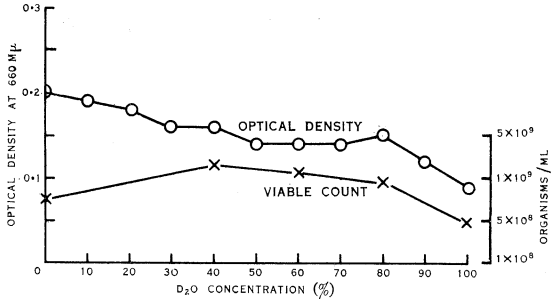

Fig. 2.—Effect of D_2O concentration on total and viable count of *Serratia marcescens*.

(*a*) *Pigment Production on Solid Media*

Suspensions of *S. marcescens* were plated out on the surface of Difco nutrient agar plates containing 0, 40, 60, 80, and 100% D_2O respectively so that each plate contained about 200 colonies. The plates were incubated at room temperature in dry air and examined daily for 7 days. On the 100% D_2O medium the colonies were unpigmented though transfer back to light water media showed that they still retained

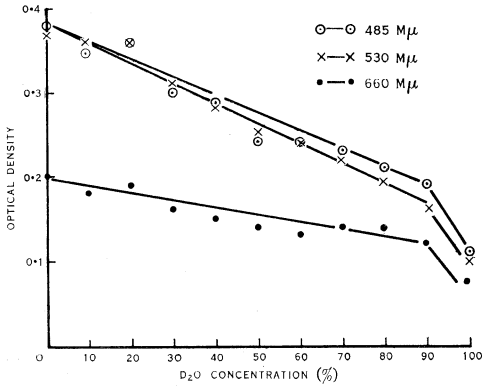

Fig. 3.—Optical density measurements at 485(⊙), 530(×), and 660(●) mμ of *Serratia marcescens* broth cultures.

the ability to form pigment. On the media containing intermediate concentrations of D_2O the colonies produced less pigment, but all the colonies on a plate were pigmented to the same extent. This result suggests that the impaired pigment production in the presence of D_2O is not due to the production of mutants.

At this stage other effects of deuteration were looked for and the appropriate dehydrated media were reconstituted in both light and heavy water and inoculated.

We found that the organism grown in heavy water gave a similar biochemical pattern to the one outlined at the beginning of this paper, except that acetyl methyl carbinol production could not be detected. This aspect was further investigated and a culture was left in contact with 100% D_2O for 12 days. This was compared with a similar culture of the organism which was grown in light water and diluted with water immediately prior to testing so that a similar optical density reading was obtained. In both cases the presence of pyruvic acid was demonstrated, though only the diluted light water culture gave a positive test for acetyl methyl carbinol. Residual glucose estimations were carried out, but in both cases all the available glucose had been used up.

IV. Discussion

Borek and Rittenberg (1960) reviewed the work done up to that time and said "A variety of effects on growth have been observed in the presence of excess D_2O in living cells. These have been invariably attributed to the toxicity of deuterium".

As a result of their own experiments Borek and Rittenberg challenged this concept, claiming that deuterium was no more toxic than hydrogen, that the anomalous growth effects were "caused not by the rare isotope per se but rather by the unusual mixture of isotopes within the organisms when they are transferred from the environment of one isotope to another." From the work reported in the present paper it appears that the true picture is rather a combination of these two conflicting views. The suppression of pigment production of *Serratia* appears to be a permanent effect of deuteration which is reversed immediately on transfer back to a light water medium. Furthermore, the experiments on glucose metabolism indicate that fully deuterated *Serratia* does not metabolize in the same manner as the organism grown in light water.

We suggest that the effects of deuteration on bacteria are of two types, transient deuteration effects such as increased lag phase and morphological abnormalities, and permanent deuteration effects such as increased generation time and altered metabolic pathways. At least this would appear to be the case with *Serratia* with its progressive suspension of pigment production and its failure to produce acetyl methyl carbinol.

We suggest that the transient deuteration effects are those referred to by Borek and Rittenberg (1960) which are produced by the sudden change of isotopic environment and the permanent deuteration effects can fairly be ascribed to the "toxicity" of deuterium.

V. Acknowledgment

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VI. References

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