I am grateful to Dr. C. E. Dalgliesh for the gift of an authentic sample of *ortho*-tyrosine. This investigation into the amino-acid metabolism of a developing insect cuticle is being continued, and a full account will be given in due course.

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Inhibition of Luminescence by Sodium Diethyldithiocarbamate and its Recovery by Sodium Borate in Luminous Bacteria

RECENTLY, significant roles of heavy metals which affect pigment formation in various animals have been examined in this laboratory[1,2].

I have investigated the effects of various metals and metal-chelating compounds on the luminescent reaction in a luminous strain, *Photobacterium phosphoreum*. The bacteria were cultured for 18 hr. in the nutrient medium at 25° C. Cells were harvested by centrifugation and suspended in 3 per cent sodium chloride solution. This suspension emitted a very brilliant light continuously for several hours, but if sodium diethyldithiocarbamate solution was added to this suspension (final concentration 10^{-3}–10^{-4} M), the luminescence ceased or decreased rapidly in a few seconds.

Among many kinds of metal-chelating compounds thus far tested, namely, sodium diethyldithiocarbamate, diphenylthiourea, ethylenediamine tetra-acetate, 8-oxyquinoline, phenylthiourea, thiourea and thiouracil, the first was the most effective in stopping the bacterial luminescence. This inhibitory effect was eliminated by addition of certain metal solutions such as ferric chloride, cupric chloride, zinc chloride and sodium pyroborate.

Among the metal solutions thus far tested, sodium pyroborate solution was the most effective in the recovery of luminescence, and boric acid was as active as 60 per cent of sodium pyroborate in an equimolal concentration. Sodium diethyldithiocarbamate solution to which sodium pyroborate was added previously has little effect on the luminescent reaction.

Sodium diethyldithiocarbamate solution has two absorption maxima at 255 mμ and 280 mμ respectively; but these peaks disappear when cupric chloride is added to the solution. On the other hand, sodium pyroborate shows no effect on the elimination of these peaks. Therefore, the concentration of sodium diethyldithiocarbamate may be estimated spectrophotometrically by examination of these absorption peaks in the case where sodium pyroborate is used.

Equal volumes of luminescent bacterial suspension were put into four tubes. Sodium diethyldithiocarbamate solution was added to the first and second tubes so that the final concentration was equal. After the luminescence of both tubes disappeared, sodium pyroborate solution was added to the first tube and the same volume of distilled water was added to the second tube as a control. The luminescence of the second tube was recovered as described before. Then the bacterial cells were removed from these four tubes by centrifugation.

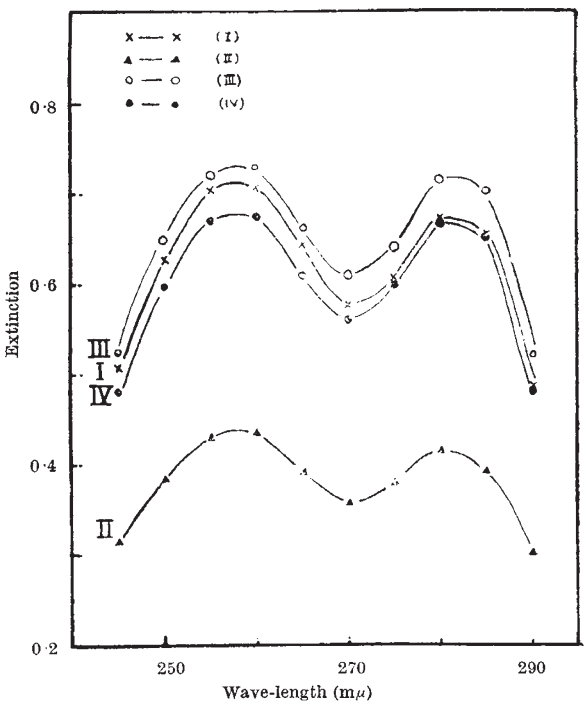

Fig. 1. Absorption spectrum of sodium diethyldithiocarbamate in the supernatant of the first tube (I), the second tube (II), the third tube (III) and the fourth tube (IV). The concentration of the carbamate and sodium pyroborate used is 10^{-4} M

After centrifugation, sodium diethyldithiocarbamate solution was added to the third and fourth tubes so that the final concentration was the same as before. Then sodium pyroborate solution was added to the third tube and the same volume of distilled water was added to the fourth tube.

These supernatants were assayed spectrophotometrically. The result (Fig. 1) shows that sodium diethyldithiocarbamate concentration in the supernatant layer of (II) is lower than that of (IV), but sodium diethyldithiocarbamate concentration in the supernatant layer of (I) is nearly equal to that of (III). This means that sodium diethyldithiocarbamate which is added to the bacterial suspension is absorbed by bacterial cells and removed with them by centrifugation, but it is liberated from the cells into the supernatant layer when sodium pyroborate is added.

The luminescent bacterial cell to which sodium pyroborate was previously added was not affected by the subsequent addition of sodium diethyldithiocarbamate; but the effect of this compound was as active as the untreated cells when the cells were centrifuged and freed from sodium pyroborate prior to the addition of sodium diethyldithiocarbamate.

Judging from these results, it may be assumed that the recovery of luminescence by the sodium pyroborate is due to the removal of sodium diethyldithiocarbamate from the bacterial cells.

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