THE DEHYDROGENASE ACTIVITY OF RESISTANT STRAINS OF \textit{ESCHERICHIA COLI} TO STYLOMYCIN AND VIOMYCIN

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INTRODUCTION

When a normal culture of \textit{Esch. coli} is passaged in medium containing increasing concentrations of Stylomycin and Viomycin, resistant strains of the organism develop. This phenomenon of adaptative response thus obtained has been explained (Davies and Hinshelwood, 1943, 1944; Hinshelwood and Lodge, 1944) to be due to the enzyme balance of the cells. Various properties of resistant strains have been studied (Sharma and Shukla, 1957, 1958, 1960). The authors (Nigam and Shukla, 1961, 1962) have studied resistance of \textit{Esch. coli} towards Stylomycin with respect to lag phase, average velocity coefficient, mean generation time, oxygen consumed and carbon dioxide evolved. The catalase activity of \textit{Esch. coli} in Viomycin resistant strain has been reported by Shukla and Nigam (1962). The effect of adaptation on activity of the dehydrogenases of \textit{Bact. Lactis aerogenes} with different carbohydrates and inhibitors in the medium has been studied (Davies and Hinshelwood, 1947).

In the present communication the dehydrogenase activity of the normal and the resistant strains of \textit{Esch. coli} to the action of Stylomycin and Viomycin has been studied and compared.

EXPERIMENTAL

The strain of \textit{Esch. coli} (faecal type 1–50/1–2) was grown in the medium (Green and Sevag, 1946) with the following composition:

- Ammonium monohydrogen phosphate \ldots 2 g./L.
- Potassium hydrogen phosphate \ldots 2 g./L.
- Glucose \ldots 2 g./L.
- Sodium chloride \ldots 4 g./L.
- Magnesium sulphate \ldots 100 mg./L.
- Calcium chloride \ldots 100 mg./L.
- Ferrous sulphate \ldots 100 mg./L.
Ferrous sulphate was omitted and pH adjusted at 7.2. Calcium chloride was sterilized separately and added in the cold to prevent precipitation. The fermentation was allowed to proceed at 37° ± 0.1° C.

The training of the organism to the drugs was effected by serial passages with increasing concentration of the drug ranging from 50-500 mg./L. Finally the culture was plated in drugged medium (500 mg./L.) and the colonies picked up after twenty-four hours and inoculated in liquid drugged medium (500 mg./L.).

500 ml. of the medium was allowed to ferment and cells harvested after 18 hours and washed three times with 0.8% sodium chloride solution. The cells so obtained were shaken for twenty minutes to obtain a homogeneous suspension and diluted to obtain a turbidity of 600 μg./ml. of dry weight of bacteria, by noticing the optical density on Spekker's absorptiometer.

Since interest centred in the enzyme activity per cell under culture conditions, intact organisms were used. Thumberg Technique as described (Umbreit, Burris and Stauffer, 1959) and applied by Quastel (Quastel and Whetham, 1924) was employed. For the determination of rate of reduction of methylene blue Thumberg tubes with hollow stoppers were used. In the main tube 2 ml. of phosphate buffer at pH 7.4, 2 ml. of 0.02 M glucose and 0.1 ml. of methylene blue (0.04%) were added. One ml. of the above suspension was put in the hollow stopper. The tubes were evacuated and equilibrated in a thermostat at 37° ± 0.1° C. After 10 minutes the tubes were tilted so as to mix the solution and the culture and the reduction rate of methylene blue was noted by Klett's absorptiometer by reading the dial from time to time. A control tube having no glucose was also taken, which showed no reduction during our experiments. Each experiment was conducted in triplicate and the average taken.

The concentration of dehydrogenase was computed according to the reaction:

\[
\text{Substrate + Carrier = Reduced Carrier + Oxidised Substrate} \\
\text{Reduced Carrier + Methylene blue = Carrier + Reduced methylene blue.}
\]

The extent of methylene blue reduction was measured in terms of absorption in Klett's absorptiometer at a uniform time interval of 30 minutes. [If 'a' was the reading at 0 hour and 'x' the reading after time 't' ranging from 5-30 minutes, (a - x) was taken to represent the amount of standard methylene blue reduced and plotted as the ordinate.]
(Stylomycin used was a product of Lederle Laboratory, U.S.A., and Viomycin used was of Parke, Davis & Co., Detroit, Michigan, U.S.A., sold as Viomycin Sulphate.)

RESULTS

Initial experiments were carried out to fix up the concentration of glucose to be used. It was found that 0.02 M glucose gave best results.

Figure 1 shows the reduction of methylene blue with varying pH wherein it is found that most rapid reduction took place in the range of pH 7.2 and 7.5. Subsequent experiments were, therefore, conducted at pH 7.4 using phosphate buffer.

![Figure 1](image)

**FIG. 1.** Effect of pH on reduction of methylene-blue by Normal culture of *Esch. coli*.

Figure 2 shows the reduction of methylene blue after 30 minutes of the reaction at pH 7.4 with 0.02 M glucose with varying cell mass of the bacteria as described earlier. The reduction of methylene blue was found to be directly proportional to the bacterial cell mass.

Figure 3 shows the rate of reduction of methylene blue by normal strain of *Esch. coli* (A), the same strain made resistant to Stylomycin (B) and
Viomycin (C). It is observed that there is no appreciable change in the dehydrogenase activity of the cells of the normal strain (A) and that of the Viomycin (C) resistant strain.

![Graph showing the effect of cell mass of bacteria on methylene blue reduction.](image)

**Fig. 2.** Effect of cell mass of bacteria on methylene blue reduction.

The dehydrogenase activity in case of Stylomycin strain (B) is suppressed very appreciably under identical conditions.

**DISCUSSION**

The methylene blue reduction with intact cells as in our case has not been found to be strictly a linear function but logarithmic (Tam and Wilson, 1941). The figures clearly show that the cells oxidise the glucose under normal conditions more efficiently. When the normal cells of the parent strain are made to undergo passages in medium containing Viomycin or Stylomycin they get adapted and can survive much higher concentrations of the drug over the lethal dose. The dehydrogenase activity of such adapted cells seems to behave differently with Viomycin and Stylomycin.
Dehydrogenase Activity of Resistant Strains of Esch. coli

Davies and Hinshelwood (1947) have shown a definite correlation between adaptation and power of oxidisation of the substrate wherein adaptation seems to confer the ability to oxidise the carbohydrates in a specific manner giving preference to those which are easily oxidisable. The enzymes are effected, in a partial manner at least, by the drugs which in some way retard the enzymes in their oxidation reduction pathway and this retardation differs markedly in Viomycin and Stylomycin.

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**SUMMARY**

The strain of *Esch. coli* has been made resistant to Stylomycin and Viomycin, wherein there is slight fall in the oxidizing power of glucose in case
of Stylomycin and nearly no change in Viomycin resistant culture when compared with the oxidising power of normal culture.

REFERENCES

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