Metabolism of 3-deoxy-3-fluoro-D-glucose by *Pseudomonas aeruginosa*

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As part of a program of investigation concerned with the metabolic effects of monofluorinated deoxysugars on yeast (Woodward, Taylor & Brunt, 1969) and certain micro-organisms, including *Pseudomonas fluorescens* (White & Taylor, 1970), we have investigated the effect of 3-deoxy-3-fluoro-D-glucose (3 FG) on *Pseudomonas aeruginosa.*

P. aeruginosa was incubated in a mineral salts medium with glucose, glucose + 3 FG, and 3 FG as carbon sources at 37° on an orbital shaker. At first growth only occurred where glucose was present as the carbon source, but subsequently the organism was induced to grow on 3 FG as the sole carbon source. Subsequent experiments showed that growth rates, utilisation of carbon source, and final cell density were similar for both glucose and 3 FG. In addition, after an initial lag, fluoride ion (F^-) was released quantitatively. 3-deoxy-3-fluoro-D-gluconic acid (3 FGA) and 3-deoxy-3-fluoro-2-keto-D-gluconic acid (3 F2KGA) also served as sole carbon sources, F^- being quantitatively released after a lag period. No F^- was detected when *P. aeruginosa* was incubated with β-fluoro-pyruvate.

Oxygen uptake was studied using Warburg respirometers (Umbreit, Burris & Stauffer, 1964) and it was found that during the lag phase when F^- was not released two atoms of O_2 were consumed per mol of 3 FG and one atom of O_2 per mol of 3 FGA ozidized.

Using Eastman precoated silica gel t.l.c. sheets and a solvent system composed of acetic acid–ethyl acetate–water (3:3:1), 3 FGA was detected in the concentrated culture filtrate of lag phase 3 FG cultures. The spots were visualized with *p*-anisidine. 3 F2KGA was not identified positively using this system, but previous work with gas-liquid chromatography has suggested that it was present in trace amounts.

Work with fractionated, disrupted, cell suspensions (Watkins, 1970) showed that the cell envelope fraction released F^- from 3 FG and 3 FGA at approximately ten times the rate as did the cytoplasmic fraction.

These results suggest that *P. aeruginosa* metabolized 3 FG by the Entner-Douderoff pathway in a similar manner to glucose. The oxidation proceeded as far as 3 F2KGA with the consumption of two O_2 atoms per mol of 3 FG, at which stage the F^- is lost when the 6 carbon compound is cleaved to two 3 carbon compounds. The enzyme system responsible for the C–F bond cleavage is probably located in the cytoplasmic membrane.

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The inactivation of phenylmercuric nitrate by sodium metabisulphite

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During work in this School concerning the uptake of phenylmercuric nitrate from solution by rubber closures, a greater rate of fall in phenylmercuric nitrate concentration was noted in the presence of sodium metabisulphte than in its absence. Aqueous solutions containing both phenylmercuric nitrate and sodium metabisulphite were therefore examined to assess any effect the latter substance might have on the phenylmercuric nitrate concentration under normal conditions of storage and sterilization. Phenylmercuric nitrate concentrations were determined by a polarographic method (Porter, 1968); antibacterial activity was monitored by the cup plate method with *Staphylococcus aureus* (N.C.T.C. 7447), as test organism.

Storage experiments. Ampoules containing a solution of phenylmercuric nitrate (20·0 μg/ml) with sodium metabisuphite (1·0 mg/ml) were kept at laboratory temperature and assayed weekly. Both phenylmercuric nitrate content and antibacterial activity fell steadily;

after five weeks the phenylmercuric nitrate concentration had fallen to 10·0 μg/ml with a corresponding reduction in antibacterial activity. Control solutions containing phenylmercuric nitrate only, showed no reduction in concentration or activity.

Heat experiments. Ampoules containing a solution of phenylmercuric nitrate (20·0 μg/ml) with sodium metabisuphite (1·0 mg/ml) were assayed after (a) exposure to a temperature between 98° and 100° for increasing periods of time and (b) autoclaving (115°, 30 min). The phenylmercuric nitrate concentration in (a) fell rapidly; after thirty minutes only 5·0 μg/ml remained and the antibacterial activity had diminished. In case (b) the presence of neither phenylmercuric nitrate nor antibacterial activity could be demonstrated. Controls in both cases showed no reduction in concentration or activity.

The use of phenylmercuric nitrate as a preservative has been criticized on other grounds (Brown & Norton, 1965; Norton, private communication), and the above results give further weight to the argument for its discontinuance. More disquieting, however, were the results obtained in examining heat-sterilized official eye drops and injections formulated to contain both phenylmercuric nitrate and sodium metabisulphite. Whether prepared at this School or purchased from reputable manufacturers, only negligible amounts ($<1{\cdot}0$ μg/ml) of phenylmercuric nitrate could be detected, and no antibacterial action could be demonstrated.

No official test is specified to ensure the antibacterial activity of added preservation in eye drops or injections. This may explain why the inactivation of phenylmercuric nitrate by sodium metabisulphite has not hitherto been reported.

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Progress towards a standard to limit particulate contamination in intravenous fluids

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Instrumental methods for detecting unwanted undissolved solid particles in intravenous solutions are more objective than the visual inspection methods at present in use.

Following the observation that there is usually a log-log relation between particle size and cumulative number, Groves (1969) proposed a standard written in the form-specific value (S) = (log $N_{1{\cdot}0}$–2·5)/M, where $N_{1{\cdot}0}$ is the estimated number of particles at a threshold of 1·0 μm, and M is the slope of the log-log distribution. This takes into account the fact that the numbers of particles and the slopes of the distributions varied widely from container to container, even those from the same batch, and is not confined to one instrumental method or principle of detection.

The response of a number of Coulter machines to both a square wave generator and to a standardized suspension of a polystyrene-DVB latex (mean diameter 8·25 μm) was measured. The standard error was $\pm 0{\cdot}31\%$ of the mean response to the square wave generator for eleven machines, and $\pm 1{\cdot}96\%$ of the mean count on the suspension for twelve machines. Provided suitable calibration materials were available it is suggested that the main obstacles to a collaborative trial of this instrument could be overcome.

The validity of the log-log relation between the numbers of contaminating particles and particle size was confirmed up to 30 μm using a HIAC Model P305-SST Automatic Particle Counter (Carver, 1969). The HIAC instrument was unable to count at levels exceeding 3000/ml. Since, in good quality intravenous solutions encountered in practice, counts rarely exceed 50 particles per ml at a threshold of 5·0 μm (e.g. Appino & Robinson, 1969) this limitation on counting rate is unlikely to constitute a problem.

Four bottles rejected from a hospital-made batch of Sodium Chloride Injection B.P. because of the presence of visible particles were examined using both the Coulter Counter and the HIAC instruments. Coulter counts were made using the device described earlier (Groves, 1969). The bottle was then disconnected and attached to the receiver of the HIAC. Replicate counts were taken on successive 40 ml volumes until the bottle contents had been used up.