DIFFERENTIAL EFFECTS OF CHLORAMPHENICOL ON THE GROWTH AND RESPIRATION OF MAMMALIAN CELLS

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The antibiotics chloramphenicol, lincomycin and erythromycin have been shown to be specific inhibitors of mitochondrial protein synthesis in yeast, both *in vitro* and *in vivo* (Clark-Walker & Linnane, 1966, 1967; Linnane, Lamb, Christodoulou & Lukins, 1968). Yeast cells growing in the presence of any of these antibiotics can no longer synthesize the mitochondrial cytochromes a, a_3, b and c_1, but their growth in glucose medium is unaffected. The observations have been interpreted as indicating that in yeast the four insoluble cytochromes of the terminal electron transport system are synthesized by mitochondria, while protein synthesis by the cytoplasmic ribosomal system is not inhibited by these antibiotics.

On the other hand, several different explanations have been advanced to explain the toxic effects produced by chloramphenicol (CAP) in mammalian tissues. For example, Godchaux and Herbert (1966) have reported that CAP at the high level of 1 mg per ml appeared to interfere with ATP formation in intact rabbit reticulocytes, and Freeman and Haldar (1967) have claimed that at 1.9 mg per ml, CAP is a specific inhibitor of NADH oxidation by isolated beef heart mitochondria, while an inhibition by CAP of amino acid incorporation into protein by reticulocyte ribosomes led Weisberger and Wolfe (1964) to conclude that the cytoplasmic ribosomes are the site of action of CAP in mammalian tissues.

This communication reports experiments which appear to rationalise some of these apparently conflicting views. We found that high levels of CAP immediately inhibited the respiration and growth of mammalian tissue culture cells,

whereas at low levels CAP specifically inhibited the synthesis of the mitochondrial cytochromes a, a_3, b and c_1 without initially affecting cell growth or respiration.

RESULTS

HeLa cells* were grown in spinner culture in Eagle's minimal essential medium (Eagle, 1959). Growth rates were determined by cell counting and by estimation of the increase in total cell protein. Viable cells were identified by their ability to exclude Trypan Blue.

(a) Effect of chloramphenicol on the growth and respiration of HeLa cells

CAP produced two distinctively different patterns of inhibition of the growth of HeLa cells. At low concentrations (10-40 µg per ml of medium), the rate of growth of the cells was initially unaffected, but after two cell divisions growth ceased abruptly. On the other hand, at higher concentrations of CAP such as 100 µg per ml the rate of growth was reduced, and at 150 µg per ml growth was completely inhibited. After 24 hours in the presence of 150 µg of CAP per ml the proportion of non-viable cells increased from about 5% in the controls to about

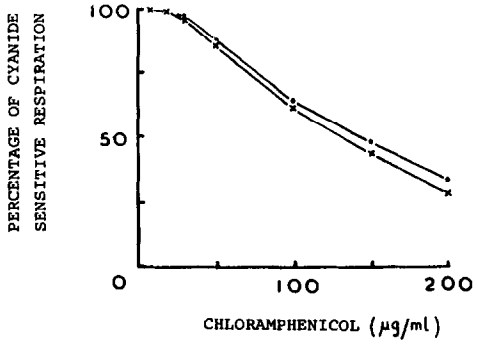

Fig. 1 The effect of CAP on the cyanide sensitive respiratory activity of intact HeLa cells, stimulated by pyruvate (2mM)•——• or succinate (5-10mM)×——×, was determined polarographically at 37°C. The respiratory rate in the absence of CAP was 0.340 µmoles $O_2/10^6$ cells/hour with pyruvate and 0.205 µmoles $O_2/10^6$ cells/hour with succinate.

* Obtained from Professor D. White, Melbourne University

30%, so that some cell death had occurred under these conditions.

Polarographic measurement of the respiration of normal HeLa cells revealed that about 25% of the respiratory activity was cyanide insensitive and this component of respiration was found to be unaffected by CAP even at concentrations of 200 µg per ml. On the other hand, although low concentrations of CAP such as 20 µg per ml had no effect on the respiration of normal HeLa cells, 100 µg of CAP per ml inhibited the cyanide sensitive

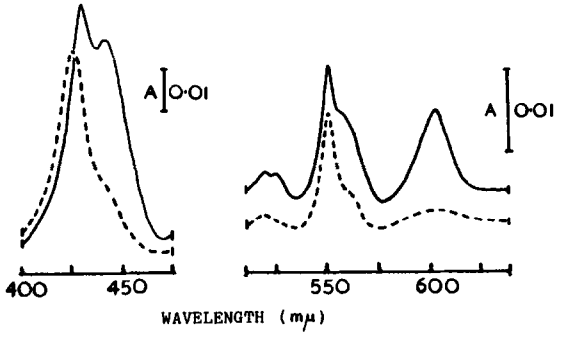

Fig. 2 Difference spectra of isolated mitochondria from normal HeLa cells ———, and from cells grown in 20 µg/ml CAP - - - - - . Mitochondria were prepared by the method of Kobayashi et al. (1966), with the omission of the Nagase treatment of the cells. The absorption peaks of cytochromes a and a_3 (605 and 444 mµ), and b (562 mµ) were extensively reduced in mitochondria from CAP grown cells.

component of respiration by about 40%, and 150 µg per ml inhibited by about 55% (Fig.1). Similarly, the respiration of the mitochondria isolated from normal HeLa cells and stimulated by pyruvate plus malate or succinate was inhibited by CAP, although about twice the concentration of CAP was required to produce a similar degree of inhibition of respiration to that required for the intact cells.

(b) Effect of low levels of chloramphenicol on the synthesis of mitochondrial cytochromes

Mitochondria were isolated from HeLa cells as soon as growth had become arrested following two cell divisions in the

presence of 20 μg of CAP per ml. As shown in Fig.2, the content of cytochromes a, a_3 and b in mitochondria from the CAP grown cells had decreased extensively, and the levels of cytochromes a and a_3, for example, were less than 20% of the levels in mitochondria isolated from normal HeLa cells. Low temperature spectroscopy showed that the mitochondrial content of cytochrome c had not decreased, whereas cytochrome c_1 had decreased by about the same extent as cytochrome b. This extensive loss of mitochondrial cytochromes would be expected to interfere with whole cell respiration, and as anticipated, the cyanide sensitive component of the respiratory activity of the CAP grown cells was decreased by about 80% in comparison with normal HeLa cells. The total amount of mitochondrial protein formed by the CAP grown cells was about the same as in the control cells.

DISCUSSION

Two effects of CAP on growing HeLa cells have been demonstrated. At low concentrations of the antibiotic (20 μg per ml) there was selective inhibition of the synthesis of the mitochondrial membrane-bound cytochromes a, a_3, b and c_1, and this action of CAP appeared to result in the inhibition of synthesis of only a small proportion of the mitochondrial proteins. These observations mirror the effects of CAP on the phylogenetically primitive yeast cell, and indicate that the antibiotic selectively inhibits mitochondrial protein synthesis *in vivo*. As in the yeast cell, protein synthesis by the cytoplasmic system did not appear to be directly inhibited by CAP *in vivo*, as cell growth was unaffected until two cell divisions had taken place. The abrupt cessation of growth of the HeLa cells is presumed to be a consequence of the change in the composition of the mitochondria and the resulting decrease in the respiratory capacity of the cells.

At comparatively high concentrations of CAP (100-150 μg per ml) the drug directly inhibits the respiration of isolated mitochondria as well as intact HeLa cells. The inhibition of mitochondrial respiration by CAP both *in vitro* and *in vivo* suggests that this phenomenon most likely accounts for the *immediate* inhibition of HeLa cell growth by CAP at 150 μg per ml.

Freeman and Haldar (1967) have reported that CAP is a specific inhibitor of NADH oxidation, but in the present work the inhibitory effect of the drug was not found to be specific for NAD linked substrates but appeared to be a more general one on mitochondrial respiration.

Our findings may be of relevance to the pathogenesis of the clinical side-effects of the antibiotic. The usual therapeutic regimes of CAP establish serum levels in the range of 5-20 μg per ml, and the side-effects of these levels of the drug on bone marrow (Scott et al., 1965) are probably related to an inhibition of mitochondrial protein synthesis. However, the generalised tissue toxicity observed in the Grey Syndrome, which occurs in the neonate at high serum levels of CAP (75-180 μg per ml)(Burns et al., 1959), may well be related to a direct effect of CAP on mitochondrial respiration.

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