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EFFECT OF ANTIBIOTICS ON THE BINDING OF PARGYLINE TO MONOAMINE OXIDASE IN CULTURED HEPATOCYTES

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Abstract—The laser dye rhodamine 123 has been used to establish that the binding of [^{3}H]pargyline to monoamine oxidase is a more sensitive indicator of mitochondrial perturbation than measurements of protein synthesis, secretion, or degradation. The amount of monoamine oxidase labelled depends on the antibiotic used. The labelling was considerably lower in the presence of gentamycin than in the presence of either chloramphenicol or of penicillin and streptomycin. The accumulation of gentamycin within the cells was the cause of the reduced labelling of monoamine oxidase which was not accompanied by an alteration in the metabolism of pargyline. The gentamycin effect can be prevented by incubating the cells in medium supplemented with methylamine prior to adding the monoamine oxidase inhibitor. Long term culture of cells with gentamycin can result in the cell culture medium becoming dark brown. Under these conditions the monoamine oxidase labelling is increased due to an inhibition of pargyline metabolism. The results indicate that the choice of antibiotic is important in patients being treated with both antibiotics and monoamine oxidase inhibitors.

Antibiotics are widely used in clinical practice and in cell culture to limit bacterial infection. However, some antibiotics may prove cytotoxic to eukaryotic cells [1]. In many cases the adverse effects are caused by an impairment of mitoribosomal protein synthesis. Certain antibiotics which inhibit protein synthesis in isolated mitochondria are benign to intact cells since they are unable to pass through the plasma membrane and/or the mitochondrial membranes. Some initially harmless antibiotics may prove detrimental if used over an extended period, owing to their accumulation within the cells.

Cells in tissue culture show an increased dependence on glycolysis and, although the tricarboxylic acid cycle is still present, it plays a lesser role than *in vivo* [2]. Part of this change may be due to adaptation to the *in vitro* situation; however, the presence of "protective" antibiotics could also have an effect.

In view of the above facts the present study was carried out to investigate the effect of antibiotics in common usage on a mitochondrial system in hepatocytes. Hepatocytes are non-dividing differentiated cells which are rich in mitochondria and are, therefore, suitable for studying the long term effects of antibiotics without complications from the cell cycle. The effects of antibiotics on rates of protein synthesis have been studied [3] but this is rather a gross assessment and the experiments were only conducted over a short period of time which would not enable the accumulative effects of an antibiotic to be observed. We have established conditions which enable hepatocytes to be cultured in a steady state of protein metabolism for a period of 7 days [4]. In our system the mitochondrial enzyme monoamine oxidase (MAO) has a half life of 60 hr and the general mitochondrial proteins have a turnover rate of 3–4 days [5]. These values are very similar to those measured *in vivo* [6]. Because of the slow turnover of most mitochondrial proteins, large numbers of experiments would have to be conducted to establish whether or not an antibiotic had an affect. Moreover, these measurements would not provide an early indication of mitochondrial malfunction. Therefore, we have examined the perturbation of mitochondrial function using the [^{3}H]pargyline labelling of MAO [7] as a sensitive indicator. The validity of this index as an improvement on measurements of rates of protein synthesis has been established by the use of the laser dye rhodamine 123 which is accumulated within mitochondria [8]. The results indicate that care must be taken in prescribing the joint use of antibiotics and MAO inhibitors in clinical practice.

MATERIALS AND METHODS

Chemicals. L-[4,5-^{3}H]leucine (124 Ci/mmol) was supplied by Amersham International (Amersham, U.K.) and rhodamine 123 was purchased from Eastman Organic Chemicals (Rochester, U.S.A.). Gentamycin and the penicillin and streptomycin were from Flow Laboratories, Irving (Ayrshire, Scotland, U.K.). Chloramphenicol and methylamine were obtained from Sigma (Poole, Dorset). All other reagents were as described previously [7].

Preparation and culture of hepatocytes. Hepatocytes were prepared as described previously [4, 9]. Cells were cultured on Primaria culture dishes (60 mm Falcon). The culture medium was Leibovitz L-15 medium pH 7.4 supplemented with glucose (8.3 mM), Hepes (25 mM), insulin (0.8 μg/ml), dexamethasone (1 μM), 10% (v/v) heat inactivated (56° for 30 min) newborn calf serum and antibiotic.

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The antibiotic was either gentamycin (50 μg/ml) or chloramphenicol (100 μg/ml) or penicillin (100 IU/ml) and streptomycin (100 μg/ml). Hepatocytes (2.5×10^6) were plated out in 3 ml of appropriate culture medium (see figure legends). The medium was changed after 2.5 hr and subsequently at 24 hr intervals unless otherwise indicated.

Measurement of intracellular protein synthesis and secretion. The medium was changed to leucine free Leibovitz L-15 culture medium which was then supplemented with L-[4,5-^{3}H]leucine (2.5 μCi/plate). Where appropriate rhodamine 123 was added (see figure legends). Cells were harvested at the times indicated and the culture medium retained. All samples were stored at −20°.

Measurement of intracellular protein degradation. The hepatocytes were plated out in 3 ml of leucine free culture medium pH 7.4. The antibiotic used was gentamycin (50 μg/ml). After 2.5 hr the medium was replaced by the same medium supplemented with [^{3}H]leucine (2.5 μCi/plate). The cells were cultured in this medium for 17 hr. Two washes (1 hr) were carried out with culture medium supplemented with leucine (10 mM). Where appropriate rhodamine 123 was added at the time of the second wash (see figure legends). A final change of medium (containing 10 mM leucine) was made after the second wash and this was taken as zero time. At the times indicated the cells were harvested and the medium retained for analysis.

Labelling of endogenous hepatocyte MAO with [^{3}H]*pargyline.* This procedure was carried out as described previously [7]. Where appropriate rhodamine 123 or methylamine was incubated with the hepatocytes prior to the addition of the radioactive MAO inhibitor (see figure legends).

Cell harvesting. Cells were harvested by scraping or by trypsinisation [4, 7]. Scraping was used for determination of cellular radioactivity and for enzymic analysis. Trypsinisation was used for the

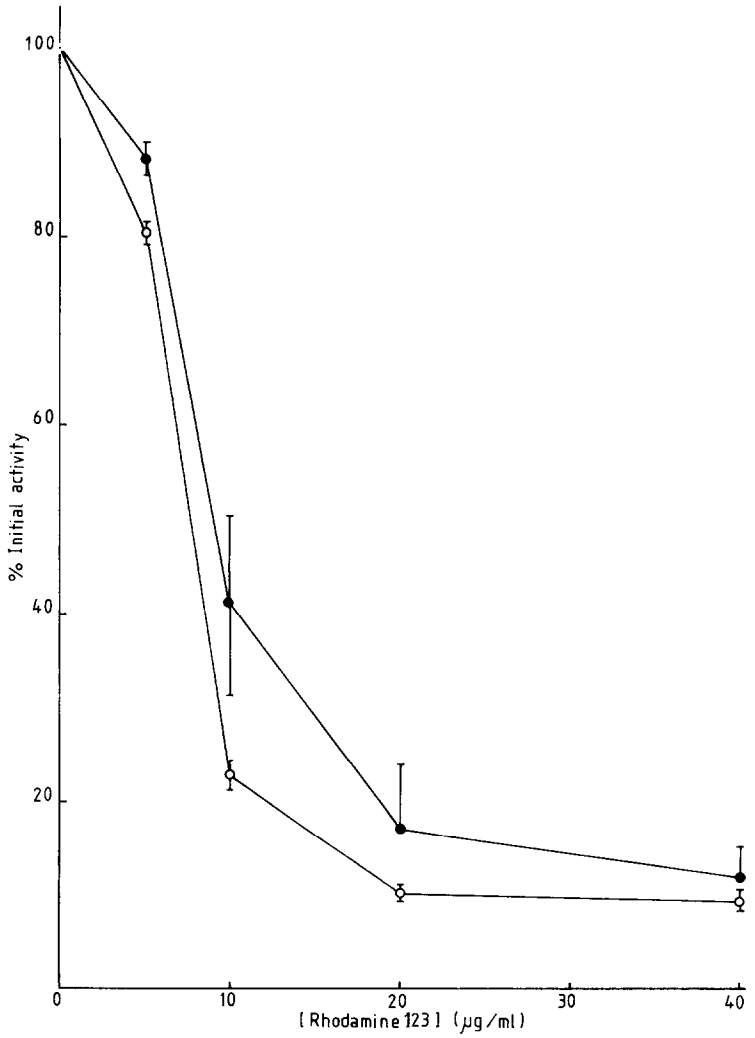

Fig. 1. Dose dependent cytotoxicity of rhodamine 123. Fresh medium supplemented with rhodamine 123 (0–40 μg/ml) was added to 24-hr cultured hepatocyte monolayers. Following a further 18 hr the cells were harvested: ○, lactate dehydrogenase activity; ●, succinate dehydrogenase activity. The values are the mean ± SD of triplicate plates.

analysis of proteins by SDS polyacrylamide gel electrophoresis. The harvested cells were stored at −20° or were used to make mitochondrial preparations.

Thin layer chromatographic analysis of [^{3}H]*pargyline metabolites.* The procedure was as described previously [7]. The solvent system was chloroform : methanol : formic acid (85 : 15 : 1 by volume).

Preparation of mitochondria from [^{3}H]*pargyline treated hepatocytes.* Hepatocytes were labelled with [^{3}H]pargyline for 3 hr. The cells were harvested and resuspended in sucrose (0.25 M) and then disrupted by sonication (1 × 10 sec burst at an amplitude setting of 4.5 μm peak to peak) using a MSE Soniprep 150. The sonicate was centrifuged twice at 500 *g* for 10 min and the resulting supernatant was centrifuged at 7500 *g* for a further 10 min. The mitochondrial pellet was retained for analysis by gel electrophoresis. Preliminary experiments, using both differential centrifugation and Percoll density gradient centrifugation, had shown that the subcellular distribution of the [^{3}H]pargyline label paralleled MAO activity. Furthermore, when compared with the distribution of the inner mitochondrial membrane enzyme, succinate dehydrogenase, it was apparent that only some 14% of the outer mitochondrial membranes had been removed from the mitochondria by the cell breakage procedure.

Analytical polyacrylamide gel electrophoresis in the presence of SDS. Gels were prepared in glass tubes (0.5 cm × 11 cm) by a modification of the method of Reisfeld and Small [10]. The running gel (8 cm long) contained 7% (w/v) acrylamide and the stacking gel contained 4% (w/v) acrylamide. The electrophoretic buffer was 0.041 M Tris/HCl (pH 8.4)/0.19 M glycine/0.1% SDS.

Mitochondrial pellets from hepatocytes labelled with [^{3}H]pargyline were redispersed in sample buffer containing Tris/HCl (0.05 M pH 6.8), SDS (10%),

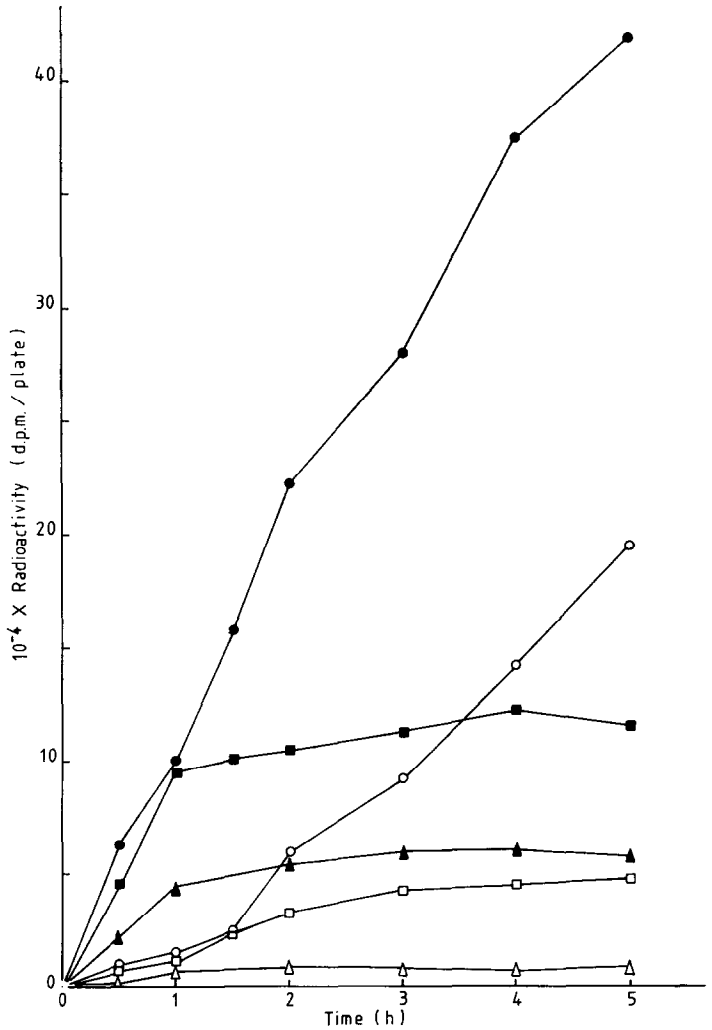

Fig. 2. Effect of rhodamine 123 on intracellular protein synthesis and secretion in hepatocyte monolayers. Hepatocyte cultures of 24 hr were used. Cells were incubated for 1 hr with rhodamine 123 prior to adding the [^{3}H]leucine. Incorporation of [^{3}H]leucine into intracellular proteins: ●, control no rhodamine; ■, rhodamine 123 (6 μg/ml); ▲, rhodamine 123 (10 μg/ml). Incorporation of [^{3}H]leucine into secreted proteins: ○, control no rhodamine; □, rhodamine 123 (6 μg/ml); △, rhodamine 123 (10 μg/ml).

glycerol (10%), 2-mercaptoethanol (10%) and bromophenol blue (0.005%). Proteins were solubilized by boiling for 2 min. Samples (100 μl) were loaded on to the gels. M_r values were determined by means of a marker kit (10,000–100,000 M_r).

Electrophoresis was carried out for between 3 and 4 hr at a constant current of 3 mA per tube. The gels were then removed from the tubes by rimming and rinsed with distilled water. The radioactivity in the gels was located by slicing them into 5-mm pieces. The sections were dissolved in H_2O_2 (500 μl 30% v/v). Formic acid (50 μl), water (500 μl) and Optiphase "X" scintillant (10 ml) were added. Radioactivity was measured with a LKB 1219 Rackbeta liquid scintillation counter.

Analyses. Samples were thawed and the cell suspensions disrupted by sonication (2 × 10 sec bursts at an amplitude setting of 6 μm peak to peak). Protein and the activities of lactate dehydrogenase, succinate dehydrogenase and MAO were measured as described previously [7]. L-[4,5-^{3}H]Leucine incorporation into intracellular and secreted proteins was determined by trichloroacetic acid (TCA) precipitation on 2.5 cm GF/C glass fibre discs [11]. [^{3}H]Pargyline associated with protein was determined [7]. TCA insoluble radioactivity in cells and the TCA soluble label released into the medium was measured as previously described [12].

RESULTS AND DISCUSSION

In a previous study [7] we reported the fate of pargyline in cultured hepatocytes. The pargyline labelling of MAO is an enzyme activated process *in vitro* [13] and would be expected to be a sensitive indicator of mitochondrial function in living cells. We have tested this hypothesis by incubating hepatocyte monolayers with rhodamine 123, which is accumulated in energised mitochondria [8]. Morita *et al.* [14] have reported that rhodamine 123, at a concentration of 40 μg/ml prevented the transport of proteins into mitochondria in hepatocyte suspension culture with-

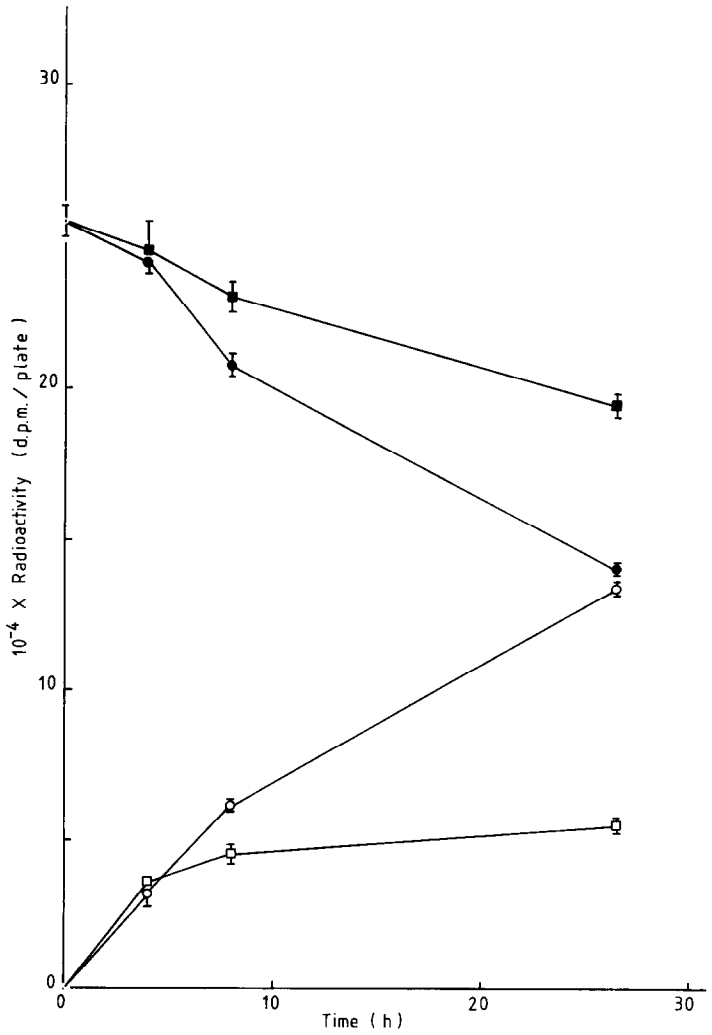

Fig. 3. Effect of rhodamine 123 on intracellular protein degradation: ●, TCA insoluble counts in cells; ■, TCA insoluble counts in cells incubated with rhodamine 123 (6 μg/ml); ○, TCA soluble counts in medium; □, TCA soluble counts in medium of cells incubated with rhodamine 123 (6 μg/ml). The values are the mean ± SD of 4 plates.

out causing a gross cytotoxic effect. However, in our monolayer cultures this concentration proved to be cytotoxic (Fig. 1). Rhodamine 123, at the concentrations used, did not inhibit the activity of either of the measured enzymes directly. The decrease in the activities of the cytosolic marker, lactate dehydrogenase, and of the mitochondrial marker, succinate dehydrogenase, were accompanied by the detachment of cells from the monolayer. We, therefore, incubated hepatocyte monolayers for 1 hr with rhodamine 123 at concentrations varying from 3 to 15 μg/ml. Subsequent morphological observations under the light microscope, on the cells in rhodamine free medium revealed that there were no visible effects up to a concentration of 6 μg/ml. Higher concentrations produced a pronounced retraction of the hepatocytes and at 13 μg/ml the cells tended to form long chains. The interaction of rhodamine 123 with hepatocytes in monolayer culture does not appear to have been reported. It is clear that the monolayers are far more sensitive to the cytotoxic action of the laser dye than are the freshly isolated hepatocytes in suspension culture. It is likely that this is due to the fact that the freshly isolated cells have atypical mitochondria with condensed cristae and thus they have a low ATP generating capacity [15]. However, in monolayer cultures the mitochondria recover from the perfusion effects in a relatively short period and ATP synthesis rises [15, 16]. The uptake of rhodamine 123 depends on mitochondrial activity [17] and the cytotoxic action of the dye is dependent on its mitochondrial accumu-

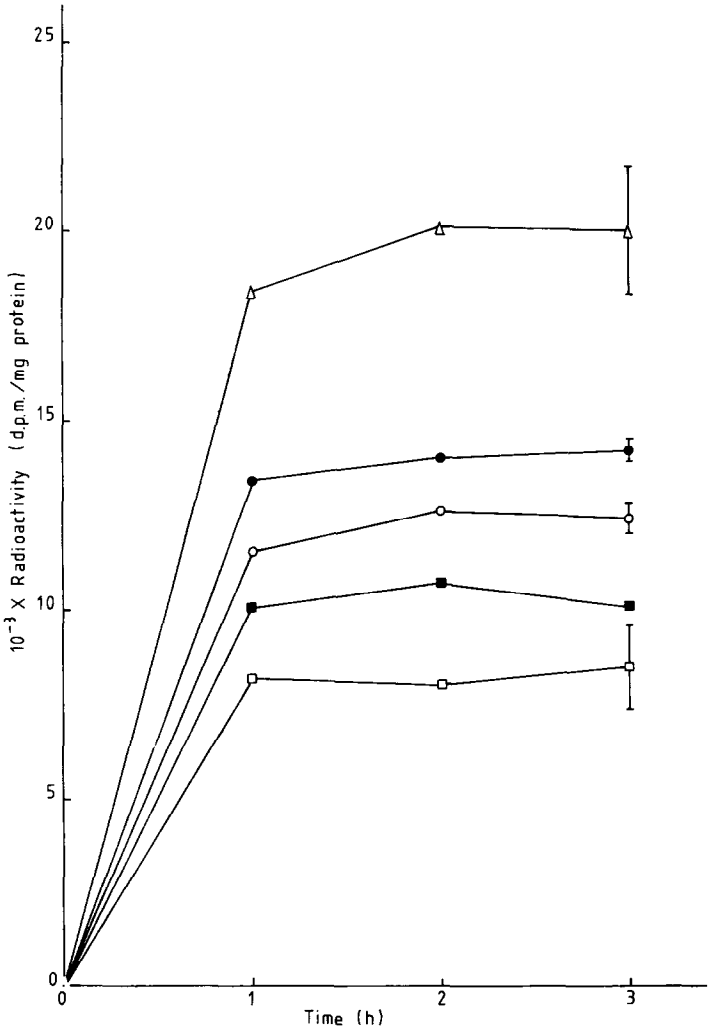

Fig. 4. Effect of rhodamine 123 on the incorporation of [^{3}H]pargyline into monoamine oxidase in cultured hepatocytes. Hepatocyte cultures of 24 hr were used. The culture medium was changed and supplemented with rhodamine 123 where appropriate. The plates were incubated for 1 hr. The rhodamine treated cells were either placed in a rhodamine free medium or left in their unchanged rhodamine containing medium. The cells were then all labelled with [^{3}H]pargyline (38 nM). Cellular TCA insoluble label: △, control no rhodamine 123 throughout; ●, incubated with rhodamine 123 (3 μg/ml) for 1 hr prior to labelling in rhodamine free medium; ■, incubated with rhodamine 123 (6 μg/ml) for 1 hr prior to labelling in rhodamine free medium; ○, incubated with rhodamine 123 (3 μg/ml) throughout; □, incubated with rhodamine 123 (6 μg/ml) throughout. The values are the mean of duplicate plates or the mean ± SD of 4 plates.

lation [18]. Therefore, it is not surprising that hepatocytes in monolayer culture are more sensitive than their counterparts in suspension culture.

Fluorescent microscopy of cells, grown as a monolayer on glass coverslips, showed that rhodamine 123 at doses of 3 μg/ml and above stained the vacuolar apparatus of the cell rather than the discrete perinuclear spots which were observed at lower concentrations. A dose of 3 μg/ml did not affect the rate of intracellular protein synthesis, secretion or degradation while 6 μg/ml drastically reduced the rate of all three processes (Figs 2 and 3). Doses of 6 μg/ml did not decrease the viability of the cells as judged by the stability of lactate dehydrogenase measurements over a 6-hr period.

The effect of rhodamine 123 on the incorporation of [^{3}H]pargyline into a TCA insoluble form is shown in Fig. 4. A dose of 3 μg/ml decreased the incorporation by 38%. The effect was most marked when the laser dye was left in the culture throughout the incubation period. Nevertheless, cells incubated at the same concentration for 1 hr and subsequently labelled in dye-free medium still showed a 29% decrease in incorporation of [^{3}H]pargyline relative to control values. Similar treatments using 6 μg/ml concentrations resulted in inhibition of labelling values of 58% and 49% respectively.

Since [^{3}H]pargyline may either be incorporated into MAO or metabolised, the influence of rhodamine 123 on the metabolism of [^{3}H]pargyline was also determined. The rhodamine 123 did not have any effect on the latter process in liver cells.

Our use of rhodamine 123 as a cellular perturbant shows that the labelling of MAO by [^{3}H]pargyline provides a more sensitive indicator of an early cell anomaly than measurements of rates of protein synthesis, secretion or degradation. Consequently, we have used this system to monitor the affect of antibiotics on hepatocyte monolayers.

The incorporation of [^{3}H]pargyline into a TCA insoluble form was unaffected by antibiotics during the first 24 hr of culture. However, by 140 hr of culture, the radioactivity incorporated in the presence of gentamycin was reduced relative to that observed in cultures supplemented either with chloramphenicol or with penicillin and streptomycin (Fig. 5). Gentamycin treatment can cause nephrotoxicity and ototoxicity but the precise mechanisms involved are poorly understood [19]. The metabolism of pargyline occurred at the same rate in all three antibiotics at 140 hr of culture. Thus accelerated metabolism in the presence of gentamycin was not the cause of the observed decrease in the labelling of MAO. The latter is a direct consequence of gentamycin accumulation within the cells. This explains an apparent paradox. In a previous report [7], using

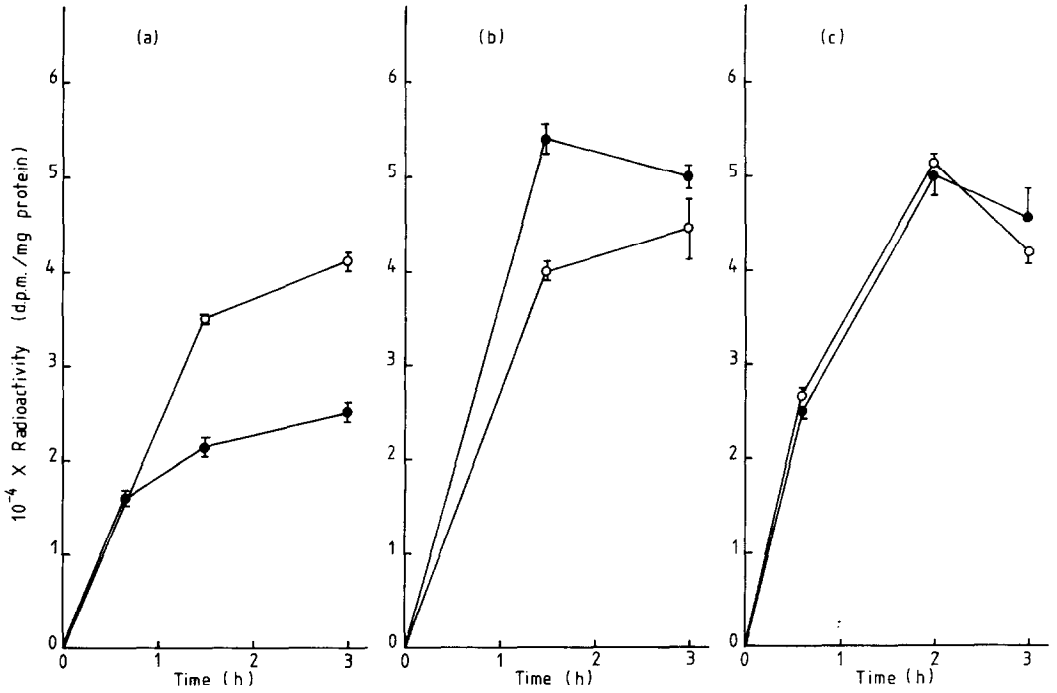

Fig. 5. Effects of antibiotics and of methylamine on the incorporation of [^{3}H]pargyline into monoamine oxidase in hepatocytes cultured for 144 hr. Hepatocyte monolayers were cultured in medium supplemented with antibiotics: (a) gentamycin (50 μg/ml); (b) chloramphenicol (100 μg/ml); (c) penicillin (100 IU/ml) and streptomycin (100 μg/ml). The medium was changed at 24-hr intervals. After 120 hr the culture media were supplemented where appropriate with methylamine (10 mM) and the cells were incubated for a further 24 hr. Finally fresh medium supplemented with [^{3}H]pargyline (38 nM) ± methylamine (10 mM) was supplied: ●, cellular TCA insoluble radioactivity; ○, cellular TCA insoluble radioactivity in the presence of methylamine (10 mM). The values are the mean ± SD (N = 3–5).

gentamycin as the antibiotic, we showed that the metabolism of pargyline decreased continuously over a 140 hr culture period but MAO labelling only increased during the first 24 hr of culture and, thereafter, remained relatively constant.

The counts incorporated into MAO after 140 hr of culture in the presence of chloramphenicol or of penicillin and streptomycin are higher than those observed in the presence of gentamycin (Fig. 5), which are the same as those measured in a previous study [7]. It is, therefore, only in the presence of the chloramphenicol or of penicillin and streptomycin that a reciprocal relationship exists between the metabolism of pargyline and its ability to label MAO.

Gentamycin does not exert its influence on 140 hr cultures by an inhibition of mitoribosomal protein synthesis. This follows since the incorporation of [^{3}H]pargyline into MAO was the same in the presence of chloramphenicol as it was in the presence of penicillin and streptomycin. The dose of chloramphenicol used effectively inhibits mitoribosomal protein synthesis in hepatocyte monolayers [12].

A decreased synthesis of MAO in the presence of gentamycin, was not the cause of the reduced [^{3}H]pargyline labelling. Although the specific activity of MAO did fall from initial values of 0.100 ± 0.010 nmol/min/mg protein (N = 12) to 0.040 ± 0.001 nmol/min/mg protein (N = 5) after 140 hr in culture with gentamycin, there was a similar fall to values of 0.050 ± 0.009 nmol/min/mg protein (N = 3) and 0.050 ± 0.007 nmol/min/mg protein (N = 5) in cells cultured in the presence of chloramphenicol or of penicillin and streptomycin respectively.

Gentamycin has been reported to accumulate in lysosomes [20], where it interferes with lipid metabolism [21]. We therefore investigated the effect of the lysosomotropic agent methylamine in the system. In 140-hr gentamycin cultures the addition of methylamine resulted in a pronounced increase in the incorporation of [^{3}H]pargyline into a TCA insoluble form (Fig. 5). The labelling attained in the presence of the lysosomotropic agent was similar to that found in 140-hr cultures supplemented with chloramphenicol or with penicillin and streptomycin (Fig. 5). Methylamine did not affect the incorporation of [^{3}H]pargyline into 20 hr cultured cells nor did it increase the level of labelling in 140 hr cultures supplemented with the other antibiotics. Finally methylamine did not affect the metabolism of pargyline under any of the conditions tested.

The increase in the incorporation of [^{3}H]pargyline into a TCA insoluble form in gentamycin cultures in the presence of methylamine (Fig. 5) was associated with a reduction of MAO activity from 91% to 74% (N = 2). This value is very similar to the MAO

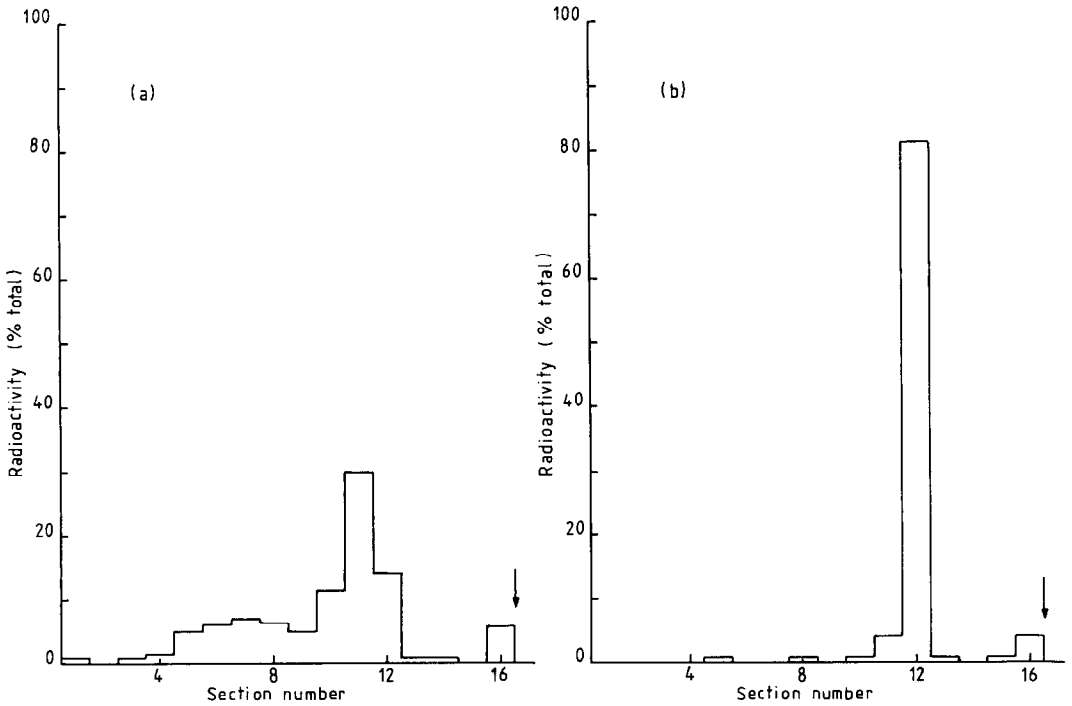

Fig. 6. SDS polyacrylamide gel electrophoresis of the radioactivity associated with mitochondria from [^{3}H]pargyline treated hepatocytes. Mitochondrial pellets were prepared from 140-hr hepatocyte cultures supplemented with gentamycin (50 μg/ml). The culture medium was changed at 24-hr intervals. At 120 hr the medium was supplemented where appropriate with methylamine (10 mM). (a) Mitochondrial pellet from gentamycin cultures; (b) mitochondrial pellet from gentamycin cultures treated with methylamine (10 mM). 320 μg of protein (specific radioactivity 26,500 dpm/mg protein) was loaded on gel (a) and 290 μg of protein (specific radioactivity 40,000 dpm/mg protein) was loaded on gel (b). The arrow indicates the position of the dye front. The recovery of the added radioactivity in the gel sections was about 70%.

activity remaining after treating 140-hr cultures with [^{3}H]pargyline in the presence of chloramphenicol (79 ± 4%, N = 3) or in the presence of penicillin and streptomycin (74 ± 5%, N = 3). This suggests that the increase in label following methylamine treatment of gentamycin cultures is due to an increased labelling of MAO rather than some non-specific effect of the agent. This was confirmed by SDS polyacrylamide gel electrophoresis (Fig. 6). Essentially all of the increased radioactivity accumulated in the presence of methylamine, was associated with a protein of MW 57,000, equivalent to the MAO subunit [22, 23].

The decrease in the amount of [^{3}H]pargyline incorporated into MAO in gentamycin cultures can be remedied by methylamine treatment. Clearly this mitochondrial system has not been irreversibly damaged by the antibiotic. The precise mode of action of methylamine is difficult to ascertain. Conventionally it is regarded as a lysosomotropic agent [24]; however, other membrane systems have been reported to swell in its presence [25]. Pargyline is a weak base and might be expected to accumulate, to a certain extent, in lysosomes. However, displacement of pargyline from, or inhibition of pargyline metabolism within, the lysosome cannot be the prime mode of action of methylamine as the latter agent is only effective in gentamycin cultures. Nevertheless, as both gentamycin and methylamine are concentrated in lysosome it is possible that the

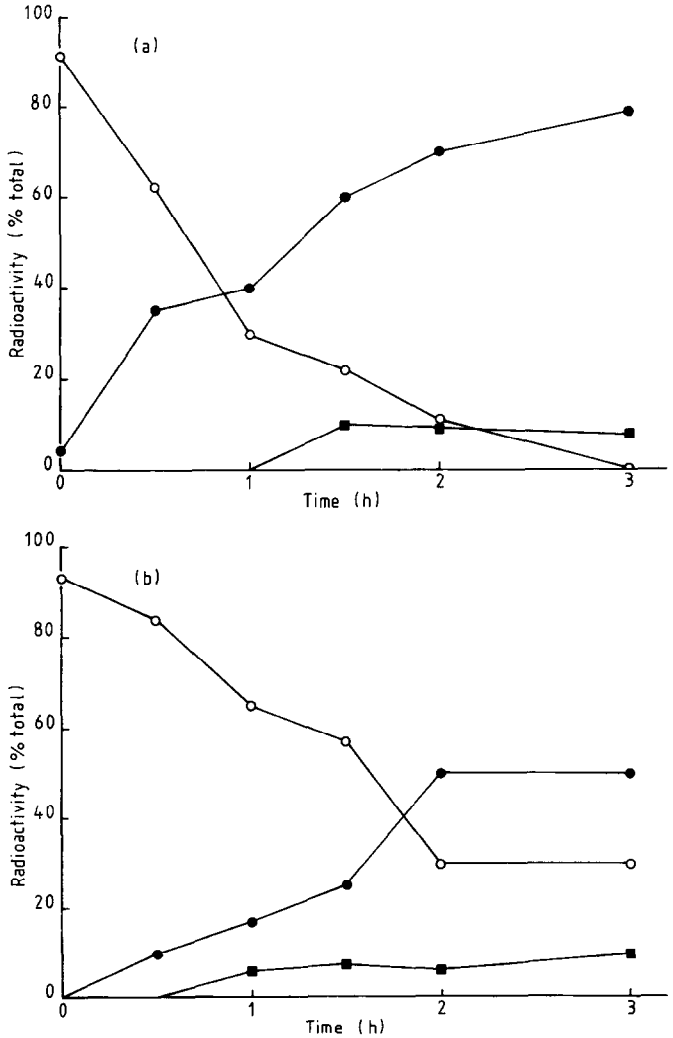

Fig. 7. [^{3}H]Pargyline metabolism in hepatocytes cultured in the presence of gentamycin for 216 hr. Hepatocytes were cultured in the presence of gentamycin (50 μg/ml) for 168 hr with 24-hr media changes. The media on the plates were subsequently either changed every 24 hr or left on the cells for a further 48 hr. The unchanged cell culture medium was dark brown at this stage. Fresh medium supplemented with [^{3}H]pargyline (38 nM) was added to both sets of cultures at 216 hr. The metabolism of [^{3}H]pargyline was analysed by thin layer chromatography. (a) Metabolism of [^{3}H]pargyline by hepatocyte monolayers (216 hr) whose culture medium was unchanged in colour. (b) Metabolism of [^{3}H]pargyline by hepatocyte monolayers (216 hr) whose culture medium had previously undergone the brown colour change. ○, pargyline (R_f 0.75); ●, metabolite (R_f 0.57); ■, metabolite (R_f 0.66). The results are the mean of 2 balanced cell culture experiments.

gentamycin and some pargyline interact in these organelles resulting in the inability of the pargyline to label MAO and that the interaction is disrupted by methylamine.

Long term hepatocyte culture in the presence of gentamycin was characterised by the development of a dark brown coloration in the cell culture medium. This phenomenon does not appear to have been reported previously. Rapid colour development occurred by using a 24-hr culture medium changing protocol for 144–168 hr followed by a 48-hr period when the medium was not changed. Colour development was dependent on the accumulation of gentamycin within cells (multiple culture medium changes) and did not occur when chloramphenicol or penicillin and streptomycin were used instead of gentamycin. Once the colour had developed a fresh medium change was still followed by its rapid reappearance. This colour was not due to a change in the pH of the medium. Difference spectra showed that the dark colour had an A_{max} at 560 nm. Thin layer chromatography in a solvent system of chloroform : methanol : formic acid (85 : 15 : 1 by vol.) revealed the presence of a new fluorescent spot with an R_f of 0.4.

After 216 hr the MAO specific activity in gentamycin treated cells was 0.020 ± 0.008 nmol/min/mg protein (N = 3) irrespective of whether the medium had changed colour or not. However, following a medium change, the [^{3}H]pargyline incorporated into a TCA insoluble form was 6063 ± 637 dpm/mg protein (N = 4) after no colour change and 14,350 ± 650 dpm/mg protein (N = 4) after the colour change. The reason for the increased counts in the latter situation was due to an alteration in the rate of metabolism of pargyline. Figure 7 shows that when the medium had remained normal in colour [^{3}H]pargyline was depleted at the same rate as had previously been observed in 140 hr cell cultures [7]. However, the cells from medium which had previously turned brown metabolised [^{3}H]pargyline at a much slower rate with some 30% of the pargyline remaining after 3 hr.

Gentamycin is routinely used in hepatocyte cultures for extended periods [26, 27]. Seglen *et al.* [3] have reported that gentamycin is the antibiotic with least effect on hepatocyte protein synthesis and degradation. However, these were short term experiments which did not allow the antibiotic to accumulate within the cells. In this report we have shown that the extent of incorporation of [^{3}H]pargyline into MAO in cultured hepatocytes can be used as a sensitive indicator of mitochondrial perturbation. We have used the laser dye rhodamine 123 as a model perturbant to show that the system is more sensitive than measurements of intracellular protein synthesis and secretion. Our system has shown that the antibiotic gentamycin is far from being benign in cell culture. It affects both the extent of mitochondrial labelling by the MAO inhibitor pargyline and can also markedly reduce the metabolism of pargyline in cultured hepatocytes. The first observation is of clinical importance. Pargyline is used clinically in the U.S.A. and in the U.K. [28]. The use of MAO inhibitors, such as pargyline, in the treatment of several disorders has proved effective. However, they have not been fully exploited owing to an association with side effects [29]. They are therefore prescribed at the minimum dose to effectively inhibit MAO [30]. In the presence of the antibiotic gentamycin a given dose of pargyline will vary in effect depending on the duration of treatment. Initially the fate of pargyline would be uninfluenced by gentamycin. However, the beneficial effects of MAO inhibitors *in vivo* are associated with their long term usage [31]. Under these conditions gentamycin would be expected to decrease the effectiveness of the MAO inhibitor and, moreover, decreased incorporation of pargyline into MAO would also cause more pargyline to be metabolised. Metabolism of MAO inhibitors is associated with tissue injury [32].

The advisability of avoiding certain foods and drugs in patients receiving MAO inhibitors is documented. In this study we show that the choice of antibiotic may also be important in patients simultaneously treated with MAO inhibitors.

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