Antibacterial drugs and their interference with the biogenesis of mitochondria in animal and human cells
A.M. KROON. AND C. VAN DEN BOGERT.

ABSTRACT
Mitochondrial RNA and protein synthesis in mammalian cells is sensitive to inhibition by a variety of antibiotics which are used in medical practice. In spite of the intrinsic sensitivity of the synthetic processes to these drugs it appears that inhibition in intact cells and living organisms is not observed in all cases because the cellular membranes may act as barriers which prevent the antibiotics from reaching their intramitochondrial targets. This holds for the rifamycins, the lincomycins and a number of macrolides but not for chloramphenicol and its analogues. Some of the toxic side-effects of the latter antibiotics can be related to their antimitochondrial action. For the tetracyclines selectivity in permeability exists in the sense that some cell types are permeable and others are not. The hypothesis is developed that the deliberate inhibition of mitochondrial protein synthesis in vivo may lead to cell proliferation arrest and offer a device in combined modality treatments of malignant growths. This hypothesis is supported by the results of two studies with experimental tumour models in rats and by retrospective and prospective clinical data.(Pharm Weekbl [Sci] 1983;5:8[-7)

INTRODUCTION
All human and animal cells except mature erythrocytes contain up to several hundred mitochondria per cell. These are the powerhouses of the cell and their main functions are related to the aerobic oxidation of substrates coupled to the phosphorylation of adenosine diphosphate to. generate adenosine triphosphate (ATP), the principal form of chemical energy for cellular labour and synthetic activities.
In the late fifties the first reports appeared indicating that mitochondria may contain their own systems for the synthesis of nucleic acids and proteins. Although data of this kind were often considered artefactual it is now well documented that mitochondria in all living organisms contain a unique genetic system. The discovery of mitochondrial DNA (mtDNA) around 1965 was one of the highlights in
establishing the role of the mitochondria themselves in their biogenetic activities. An old view that mitochondria are autonomous organelles of prokaryotic origin within the eukaryotic cell revived for some time. With the aid of the recently developed methods of gene characterization it has become clear, however, that autonomy is not to be expected.

The complete base sequence of mtDNA of man and several animals is now available. The genetic information contained in this mtDNA is too small to make the organelles autonomous within the cell. The cell is dependent on the interplay between the nucleo cytoplasmic and mitochondrial systems for the biogenesis of functional mitochondria. Neither of the systems alone is able to govern the formation of functional mitochondria (for review, see ref. 1).

THE PRODUCTS OF MITOCHONDRIAL TRANSCRIPTION AND TRANSLATION

About the organization and expression of the mitochondrial genome quite a lot of data are available, although questions about the fine regulation of the two systems are yet largely unanswered. Detailed knowledge is available about the products of mitochondria! RNA synthesis, especially for higher animals, including man (Table I).

Mitochondrial DNA codes for two ribosomal RNA's and 22 transfer RNA'S (tRNA's). These transcripts together provide all the necessary RNA's for the mitochondrial translation machinery, consisting of specific ribosomes and a full set of specific tRNA's. It is worthwhile to note that the 22 mitochondrial tRNA's are indeed able to fully decode a genetic message, albeit in a way different from the cytoplasmic system. The genetic code of mitochondria shows a few characteristics by which it deviates from what has been considered hitherto 'the universal code'.

The ribosomal proteins, the amino acid activating enzymes and protein factors for the initiation, elongation and termination of peptide synthesis are, as far as we know at present, all coded on the chromosomal DNA of the nucleus, produced on cytoplasmic ribosomes and then imported into the mitochondria. Besides the instrumental RNA's there are a number of informational or messenger RNA's. Eleven transcripts have been identified, all potentially coding for polypeptides, Two of these RNA's contain partly overlapping information for two potential polypeptide products in different reading frames.
The nature and function of the thirteen translation products is not yet elucidated for all genes (Table I). Eight reading frames are so far unassigned. The genes identified code for polypeptides which are integrated in the enzyme complexes involved in oxidative phosphorylation.

TABLE I. The products of mitochondrial transcription and translation in mammalian cells

| 16S and 12S ribosomal RNA | assembled in specific 55S mitochondrial ribosomes |
| 22 Transfer RNA'S | involved in decoding mitochondrial messenger RNA's |
| 11 Informational RNA's | corresponding to 13 open reading frames, of which 5 are assigned to known translation products and 8 as yet unassigned |
| Translation products | subunits I, II and III of cytochrome c oxidase |
| . | apocytochrome b |
| . | the 25 000 dalton subunit of the ATP-synthetase |
| . | possibly one further subunit of the ATP-synthetase |

These complexes are part of the inner mitochondrial membrane and it is thought that the characteristic demands of these membrane components form one of the reasons why nature has conserved the separate genetic system within the mitochondria. On the basis of translation data for rat mitochondria there are indications that two subunits of the ATP-synthetase complex are the products of mitochondrial protein synthesis. (3) Identification of one of the genes has not yet been achieved.

The major products of the mitochondrial translation activity form part of the enzyme complexes involved in oxidative phosphorylation. In the mitochondria these complexes, numbered I to V, are responsible for the oxidation of NADH (complex I) or succinate (complex II) via the respiratory chain (complex III or bc1 complex and complex IV or cytochrome c oxidase). The final reaction is the reduction of oxygen.

Along the respiratory chain there are three positions where oxidation is coupled to the formation of ATP (complex V for ATP-synthetase). The complexes III, IV and V contain polypeptides from both nucleocytoplasmic and mitochondrial translational origin.
As already mentioned above their formation is dependent on the activity of both genetic systems of the cell. Without the availability of the subunits made within the mitochondria, the subunits coded for by the nucleus and synthesized on the cytoplasmic ribosomes are unable to assemble into an active complex and *vice versa*. One of the most vital processes in obligatory aerobic organisms - the capability to use oxygen to provide energy for cellular processes - is thus dependent on the concerted action of the two genetic systems. The genetic information of mitochondrial DNA, albeit very little, concerns key enzymes and is therefore indispensable for aerobic cells and organisms.

**ANTIBACTERIAL DRUGS**

The possible evolutionary origin of mitochondria as descendents of prokaryotic ancestors has already been mentioned. In the early days of the investigations on the biogenesis of mitochondria, much emphasis was put on the similarities of the bacterial and mitochondrial systems for gene expression. Little by little this view had to be disregarded, especially for mammalian mitochondria. Resemblances between mitochondria and present-day bacteria are neither obvious nor numerous. Nonetheless, it remains likely that mitochondria are more related to prokaryotic microorganisms than to the nucleocytoplasmic eukaryotic system for transcription and translation. This can be concluded from comparative studies on the base sequences of nucleic acids such as the ribosomal RNA's (4). A striking and intriguing aspect of this relatedness is further found in the sensitivity of mitochondrial transcription and translation to inhibition by a variety of antibacterial drugs (Table II).

(Table II)

**TABLE II. Antibiotics interfering with mitochondrial transcription and translation in mammalian cells**

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Target</th>
<th>Special features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifamycins</td>
<td>mtRNA polymerase</td>
<td>No effect in vivo due to impermeability of mitochondrial membranes</td>
</tr>
<tr>
<td>Macrolides</td>
<td>Large subunit of mt ribosome</td>
<td></td>
</tr>
<tr>
<td>Spiramycin}</td>
<td></td>
<td>No effect in vivo due to impermeability of plasma membrane</td>
</tr>
<tr>
<td>Tylosin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Oleandomycin
Erythromycin
Carbomycin
Lincomycins
Chloramphenicol and analogues
Tetracyclines (cf Table 111)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Target</th>
<th>Effect in Vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleandomycin</td>
<td>No effect in vivo due to impermeability of mitochondrial membranes</td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Highly toxic</td>
<td></td>
</tr>
<tr>
<td>Carbomycin</td>
<td>Large subunit of mt ribosome</td>
<td></td>
</tr>
<tr>
<td>Lincomycins</td>
<td>No effect in vivo due to impermeability of mitochondrial membranes</td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol and analogues</td>
<td>Peptidyltransferase</td>
<td></td>
</tr>
<tr>
<td>Tetracyclines (cf Table 111)</td>
<td>Small subunit of mt ribosome</td>
<td></td>
</tr>
</tbody>
</table>

Rifampicine, an inhibitor of bacterial transcription also inhibits mitochondrial RNA-polymerase (5)

Furthermore, antibiotics interfering with bacterial ribosome function also inhibit mitochondrial ribosomes in spite of the fact that the physicochemical properties of animal mitochondrial ribosomes strongly differ from those of the bacterial ribosomes. This interference holds for drugs interfering via the small ribosomal subunit, for instance the tetracyclines as well as for drugs such as chloramphenicol inhibiting processes confined to the large ribosomal subunit, e.g. the peptidyltransferase activity. In practice this means that these antibiotics have an intrinsic target in the eukaryotic cells as well.

It should be realized, however, that the mitochondrial ribosomes and all other components of the biosynthetic machinery are located in the matrix of the mitochondria and thus surrounded by two biological membranes. It is well known that such membranes act as barriers with respect to the transport of all kinds of agents, including antibiotics. In fact this is the case for erythromycin, some other macrolides and lincomycins. In principle mitochondrial ribosomes are sensitive to inhibition by these antibiotics. However, they do not penetrate the mitochondrial membranes. As a consequence protein synthesis is not inhibited by these drugs in intact mitochondria, neither in vitro nor in the living animals. There are, however, members of the family of macrolides which do penetrate. Carbomycin is an example. This is a rather toxic drug that is not clinically used to our knowledge.

We have used the different behaviour of erythromycin and carbomycin to investigate the nature of the barrier in some detail. Isolated intact mitochondria incorporate amino acids into protein. This incorporation is strongly inhibited by low concentrations of carbomycin and not affected by high
concentrations of erythromycin. By combining the two antibiotics in the intubation medium we could show that the inhibition by carbomycin is considerably diminished if erythromycin is present as well (6). It has to be concluded, therefore, that the macrolides penetrate the mitochondrial membranes by means of a carrier driven mechanism. Whether the penetration is energy-dependent could not be concluded from these experiments because the test system itself, i.e. the incorporation of amino acids into protein, needs ATP.

Erythromycin does not interfere with the inhibition by chloramphenicol, whereas the inhibitions by chloramphenicol and carbomycin are additive. Thus, the presumptive carrier apparently shows specificity for the macrolides. Erythromycin, and most likely also oleandomycin, competitively block the carrier that efficiently transports carbomycin. In vivo and also with cells in tissue culture the situation with the macrolides is even more complicated. Some macrolides, e.g. spiramycin and tylosin do not penetrate the plasma membrane and will for this reason not inhibit mitochondrial protein synthesis in intact cells (6).

**CAN TOXIC SIDE-EFFECTS OF ANTIBIOTICS BE ATTRIBUTED TO IMPAIRMENT OF MITOCHONDRIAL BIOGENESIS?**

We have emphasized that energy generation via oxidative phosphorylation is guaranteed only if mitochondrial biosynthetic activities can proceed normally. Further, that the normal process may be impaired by antibacterial drugs. Therefore an obvious question, especially from a medical point of view, is whether treatment of patients with the antibiotics mentioned in Table II is harmful in the sense that the energy-generating capacity of cells and tissues is diminished during such a treatment. The answer to this question cannot be given as a mere 'yes' or 'no'. The effects depend on various circumstances.

A potent antibacterial drug is chloramphenicol. It is known to give rise to a dose-dependent depression of the bone marrow during treatment. Cases of aplastic anaemia have been reported; the frequency given varies from 1 in 4000 to 1 in 30,000 in the different reports. This form of toxicity is not clearly dose-dependent and becomes manifest long after the treatment has been ceased. With respect to the first
form of toxicity it is well-established that inhibition of mitochondrial protein synthesis precede the fall in the number of reticulocytes (7). It can be attributed, therefore, to inhibition of mitochondrial protein synthesis. The late-onset toxicity has not (yet?) been satisfactorily related to impairment of the biogenesis of mitochondria.

It is noteworthy that at the start of an antibacterial treatment all cells contain a full set of functionally active mitochondria. If mitochondrial protein synthesis is effectively inhibited i.e. if the serum and tissue levels reach the minimal inhibitory concentrations during the whole treatment then still the loss of activity will be moderate in practice because the turnover of cytoplasmic components is slow and the rate of cell division low for most tissues. After one turnover or one cell cycle the specific activity of the enzyme complexes, which depends on mitochondrial protein synthesis is still 50% of the starting value. A fifty percent or even a lower rest capacity for energy generation is not necessarily hazardous for the cells and tissues in question. The reserve capacity of the tissues may be considerable.

In conclusion one may state that very little harm is to be expected from the most commonly used antibacterial regimens, which generally last only short periods and are often not accompanied by a steady-state level of the drug at an antibacterial or antimitochondrial concentration during that whole period. For chloramphenicol one has of course to reckon with the late-onset bone marrow depression mentioned, which is not related to interference with mitochondrial biogenesis. For thiamphenicol the latter form has not been reported so far, whereas the effects on mitochondrial protein synthesis are the same for chloramphenicol and thiamphenicol. Thiamphenicol has a methylsulfonyl group substituted for the nitro group in the phenyl ring of chloramphenicol. This nitro group has been tentatively suspected to be responsible for the late-onset toxicity.

The rifamycins, the macrolides and the lincosamycins show very few toxic side-effects. The latter may give a pseudomembranous colitis, which is considered to be caused by the disturbed microbiological equilibrium of the intestinal flora rather than by a direct action on the intestinal epithelial cells. Although it is difficult and perhaps hardly appropriate to explain the absence of side-effects, it is tempting to speculate that the various permeability barriers discussed above, efficiently protect the cells
and tissues against toxic damage. An important point in this context, not yet raised so far, is whether the antibacterial and antimitochondrial actions of the various antibiotics are obtained at the same concentrations. The answer is yes. We will discuss this point in detail only for the tetracyclines, the antibiotic to which we will restrict ourselves in the further course of this article.

THE TETRACYCLINES

The tetracyclines, especially doxycycline, are frequently used in medicine. The tetracyclines have a broad antibacterial spectrum and are devoid of serious side-effects (9). Interference with odontogenesis is a drawback for the treatment of children. Furthermore nephrotoxicity may occur especially in patients with already impaired kidney function. The antibacterial action is based on inhibition of bacterial protein synthesis. The tetracyclines bind to the small subunit of the bacterial ribosomes and by doing so prevent the proper function of the ribosomes in the translation process. The various members of the family of tetracyclines differ in their lipophilic and protein-binding properties. Related to this there are differences in tissue distribution for the various tetracyclines. The minimum inhibitory concentration to fight sensitive micro-organisms is in the order of 1-3 microg/ml (10). At this low concentration mitochondrial protein synthesis is also inhibited.

In this respect tetracyclines are as potent as chloramphenicol. Contrary to chloramphenicol tetracyclines also inhibit cytoplasmic protein synthesis, (11). The latter effect is obtained only at very high Tetracycline concentrations. For some of the tetracyclines 50% inhibition of cytoplasmic protein synthesis in vitro is not even reached at concentrations up to 350 microg/ml. Such high concentrations are not available if patients that are treated with the current doses of the antibiotics. Antimitochondrial levels are reached, however, during such treatments (I2 13). In table III some data on the four most commonly used tetracyclines are brought together.

The experiments in which the inhibitory effects of tetracyclines on cytoplasmic protein synthesis were tested, have been performed with reticulocytes. In these studies we noticed that haemoglobin synthesis was impaired by various tetracyclines in lysates but not in intact reticulocytes. In tissue distribution studies in rats with oxytetracycline and doxycycline blood cells and to a lesser extent also spleen showed a low specific tetracycline content. From this observation
we tentatively extrapolated that progenitor cells of the red series are also impermeable for tetracyclines under the experimental conditions employed. Because the former experiments were performed with high doses of tetracyclines the question arose if this barrier was sufficiently strong to prevent the entrance into the cell of low amounts of tetracyclines leading to concentrations insufficiently high to impair mitochondrial protein synthesis. We therefore studied the effects of in vivo treatment of rats with oxytetracycline on the proliferation of erythroid and lymphoid cells in more detail. For obvious reasons we did these experiments under strictly controlled conditions so as to prevent high serum concentrations and tissue accumulation.

**TABLE III Comparative data of four tetracyclines: oxytetracycline (OTC), tetracycline (TC), doxycycline (DC) and minocycline (MIC)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OTC</th>
<th>TC</th>
<th>DC</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1, H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>N(CH3)2</td>
</tr>
<tr>
<td>R2, CH3</td>
<td>CH3</td>
<td>CH3</td>
<td>CH3</td>
<td>H</td>
</tr>
<tr>
<td>R3, OH</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>R4, OH</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>Partition coefficient octanol/buffer, pH 7.5</td>
<td>0.025</td>
<td>0.036</td>
<td>0.60</td>
<td>1.10</td>
</tr>
<tr>
<td>Percentage bound to plasma protein over the range 1-10 microgr/ml</td>
<td>24</td>
<td>36</td>
<td>60</td>
<td>55</td>
</tr>
<tr>
<td>Half-life on repeated dosing (h)</td>
<td>9</td>
<td>12</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>50% inhibitory concentration (microgr/ml) of protein synthesis in vitro by isolated mitochondria</td>
<td>2.3</td>
<td>2.3</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>- isolated mitochondria</td>
<td>&gt;350</td>
<td>&gt;350</td>
<td>100</td>
<td>300</td>
</tr>
</tbody>
</table>

Data taken from refs. 12, 13 and from our own experiments of the drug. Administration of oxytetracycline by continuous intravenous infusion served our purpose best (14). It was shown that proliferation of erythroid and B-type lymphoid cells is not arrested by the treatment with oxytetracycline, whereas reactions depending on proliferative activity of T-type lymphocytes were impaired (15). These results show a third type of permeability barrier: a selective barrier at the plasma membrane level confined to erythroid and...
B-lymphoid cells as far as our knowledge reaches to date. The data reviewed above clearly show the complexity of the matters arising if one attempts to search for the in vivo equivalent of a well-established in vitro action. Besides differential sensitivity of various metabolic processes and permeability barriers one has to reckon with differences concerning biotransformation, excretion rates and pathways, etc. between different species. For tetracyclines such differences certainly exist for rat and man.

**CELL PROLIFERATION ARREST BY INHIBITION OF MITOCHONDRIAL PROTEIN SYNTHESIS. A HYPOTHESIS**

In the preceding paragraphs attention was paid to the side-effects of antibiotics which act as antimitochondrial drugs as well. It was emphasized that completely blocking mitochondrial protein synthesis after one cell division leads to a 50% drop in the activity of the enzymes, which depends on this synthetic activity. In effect this means that the number of functionally active respiratory chains in a cell is halved in that case or, in other words, that the energy-generating capacity of these cells is reduced by 50%. After two cell cycles this is 25% after three 12.5% and so on. On this basis we have put forward the hypothesis that effective inhibition of mitochondrial protein synthesis may cause cell proliferation arrest by diluting out the energy-generating capacity of the dividing cells to a stage that the ATP generated by the residual functional mitochondria is not able to serve the ATP demands necessary for replication and division of cells. In fact the early-onset bone marrow depression by chloramphenicol can and should be explained in this way. The hypothesis was verified in an in vitro tissue culture experiment. In the presence of mitochondriotropic antibiotics BHK-cells as well as HeLa-cells stop multiplying after one or at most two cell divisions (16). In the strict sense of the word this means that inhibition of mitochondrial protein synthesis may lead to cell proliferation arrest. Mitochondriotropic antibiotics may, therefore, have cytostatic action.

**TUMOUR MODELS**

Although in vitro effects with cells in tissue culture are as such promising, the gap between the hypothesis and the in vivo situation had to be bridged. For this reason experiments were undertaken to search for antiproliferative effects in living, tumour-bearing animals. Such experiments should fulfil a number of criteria. Firstly, the levels of the mitochondriotropic agent used should be controllable and controlled.
Secondly, the animals should be treated for relatively long periods without considerable restraint. Thirdly, a drug with low toxicity should be chosen. Finally, the tumours should be reasonably well characterized and amenable.

To meet these aims rats were treated by continuous intraperitoneal or intravenous infusion. The infusion system was fixed on the skull (14). For the drug oxytetracycline was chosen. Concentrations of this antibiotic can be easily determined fluorometrically in serum and tissues (17). Besides, as already mentioned, tetracyclines have the advantage that they do not interfere with the proliferation of red blood cells and B-lymphocytes. We have looked for effects of continuous treatment for more than three weeks on various tissues and organs (18). At the necessary concentration of about 10 microgr per ml of serum no gross side-reactions were observed, although functional impairments of striated muscle could be observed (19) and although liver regeneration could be inhibited under extreme conditions (20).

Two tumour models have been investigated so far. In the first place effects of oxytetracycline treatment on the development of the transplantable ascitic Zajdela hepatoma were studied (21). The proliferation of these cells was arrested by amounts of tetracyclines, which only inhibit mitochondrial protein synthesis. Under these conditions the tumour cells cease dividing after a few cell generations. This event was preceded by reduction of the cytochrome c oxidase activity. After six days of treatment the number of ascitic tumour cells in the treated animals was less than one third of that found in the controls infused with saline. Besides, the specific activity of cytochrome c oxidase per fixed number of cells was only one fourth of the control value in the cells of animals treated with oxytetracycline. This unequivocally shows that there is not only proliferation arrest, but also that the cells in the treated animals are deficient in functional respiratory chains and as a consequence most likely also in their energy-generating capacity.

As a second model studies were undertaken with a subcutaneously propagated Leydig cell tumour (22). In the first place it was investigated whether the tumours would strike equally well in control animals as in animals getting an oxytetracycline treatment for three weeks either from the onset of inoculation or starting up to seven days after inoculation. Only 12% of the treated animals developed a tumour during that period versus 85% in the control group. Furthermore
the growth of this tumour, once developed, could be stopped by oxytetracycline treatment. Since growth resumed after termination of the treatment we conclude that oxytetracycline acts as a cytostatic and most likely not as a cytocidal drug in this experimental system.

RETROSPECTIVE AND PROSPECTIVE CLINICAL OBSERVATIONS

A crucial point in judging the above experimental data from a medical point of view forms the utility of such data in clinical practice. The fact that tetracyclines are and have been widely used offers the possibility to search for effects of tetracyclines hitherto not recognized as beneficial for patients suffering from a malignant disease. In a retrospective clinical study it was investigated if addition of tetracyclines to various regimens of combination therapy had influenced the survival time in patients with a tumour of the nasopharynx or larynx. The study comprised 218 patients who were first admitted to the hospital and died within the period 1964-1976 (16). The patients who received tetracyclines appeared to have lived longer. From analysis of all patients from one to ten years after treatment, it further appeared that addition of methotrexate to conventional therapy (surgery, radiation) had worsened the prognosis, in contrast to tetracycline. Tetracycline alone gave slightly better results than in the control groups. It had also considerably improved survival in patients who had also received methotrexate (16). On this basis a modest prospective clinical study was started. Patients first admitted to hospital with a tumour of the nasopharynx or larynx were treated with 100 mg doxycycline orally, with 12-hours' intervals during 5-13 days. A biopsy was taken before the treatment started and on the last day. At this point the final therapy, in all thirteen cases surgery, was started. It appeared that the second biopsy material contained a significant level of doxycycline; the level was of the order (10-20 microgr/g wet weight) specifically inhibiting mitochondrial protein synthesis. The mitotic indices of the biopsy pairs were measured. There appeared to be a significant difference (23). Although the method has its limitation, the observations certainly warrant further research along these lines. In the same trial seven patients were treated preoperatively with erythromycin instead of
doxycycline and eight patients received no preoperative antibiotic treatment at all. The paired biopsies of the erythromycin-treated patients showed no differences in the mitotic indices. Of the untreated patients there was no biopsy available on admission. In the meantime the two-years' survival data of this trial are available. In the doxycycline-treated group there are ten survivors, in the erythromycin-treated group none and in the group without preoperative drug treatment two. We tend to conclude, therefore, that the inclusion of doxycycline treatment in the combined modality treatment of squamous cell carcinoma of head and neck may offer a prognostic advantage.

CONCLUSION

The results of the in vivo and in vitro experiments in combination with the data of the retrospective and prospective clinical studies, strongly favour the concept that tetracyclines may interfere with tumour cell proliferation. Furthermore, all information gathered up to now is in agreement with the hypothesis that this antiproliferative effect occurs secondary to the inhibition of mitochondrial protein synthesis. This means that it is worthwhile to consider if tetracyclines as such or in combination with other drugs or treatments may be of use in the fight against cancer in man and to investigate if there are tumour cell types which are especially vulnerable to tetracyclines. Solid tumours for which there is no satisfactory treatment as yet should be looked at with preference. Of course there remain a number of questions. Are tetracyclines killing cells or not; are tetracyclines cytostatic or cytotoxic? Are tetracyclines active as such or do tetracyclines create conditions which favour the spontaneous or iatrogenic improvement or cure of patients with neoplasm? In this respect several speculations can be made. In the first place it may be envisaged that the antiproliferative effect of tetracyclines as such slows down tumour growth to a rate that enables animals and man to fight the tumour cells with their own defence mechanisms. In the second place various other mechanisms may be operative during or after tetracycline treatment, which improve the odds for other treatments. For instance, tetracyclines may give growth synchronization of cells by blocking the tumour cell cycle at a given stage and thus enhancing the number of cells vulnerable to either radiation or chemotherapy. Further, if the energy-generating
capacity of tumour cells bad fallen drastically DNA-repair mechanisms may be inhibited and thus radiation damage may be more effective. A limitative enumeration of the hypothesis' benefits falls outside the scope of this review. Anyway, it is clear that all possibilities mentioned above can be tackled experimentally and in clinical trials. The results of these experiments and trials will show if the mitochondrial genetic system can be fruitfully used as a target in the chemotherapy of cancer in man.

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REFERENCES
12 Green R, Brown JR, Calvert RT. The disposition of

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