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Review article

The prophylactic use of antibiotics in cell culture

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Abstract

This article describes the historical development of the prophylactic use of antibiotics in cell culture as well as their effects on cells. The influence of antibiotics on cell morphology, cellular degeneration and cell death and cellular function is summarized. Cellular DNA as well as protein synthesis are affected which can lead to interference with, or even changes in, metabolic processes. Such effects must be considered in cell culture research. As antibiotics are used in multifold ways, the otherwise standardized conditions in cell culture are no longer comparable. The prophylactic use of antibiotics is rejected for scientific reasons.

Introduction

The beginning of reproducable cell culture technique dates to the year 1907, when Harrison cultivated small pieces of nerve fiber tissue from frog embryos for several weeks under aseptic conditions (Harrison, 1907).

One of the predominant problems in culturing cells *in vitro* was to avoid microbiological contamination. However, by using aseptic techniques cell lines could be cultivated for several years (Ebeling, 1919, 1922; Carrel, 1924).

Carrel (1924) was first to demonstrate that animal cells could be cultured *in vitro* without antibiotics. The sterile technique applied by Carrel was very complex and deterred many biologists from attempting cell culture methods. The introduction of antibiotics and the use of clean benches led to a sudden expansion of cell culture methods.

Initial publications (Table 1) described the harmlessness of antibiotics in cell culture. Consequently the prophylactic use of antibiotics spread widely. However, it should be noted that those studies considered only such rough parameters as cell growth and toxicity.

Today, antibiotics such as penicillin, streptomycin, neomycin, kanamycin and gentamycin as well as the antimycotics amphotericin B, fungizone and nystatin are *prophylactically* used in cell culture. For that reason the therapeutical application of other antibiotics may be neccessary for resistant germs. In instances of contamination with mycoplasms, the antibiotics tylosin, tiamutin or ciprofloxacin were used therapeutically. Whereas the therapeutical application of antibiotics is calculated, the prophylactic use of antibiotics risks neglect of sterile technique. Moreover, antibiotics may have side effects on the cells. Consequently, the prophylactic use of antibiotics may effect the results of experiments.

Standardization of cell culture conditions

Early work with cell culture techniques used biological but undefined media such as plasma clots and embryonal and tissue extracts (Burrows, 1910; Carrel, 1912, 1913; Carrel and Ebeling, 1922; Hetherington and Craig, 1939; Drew, 1927; Jakoby, 1936; Hoffmann and Doljanski, 1939; Fischer, 1941; Doljanski and Hoffmann, 1943; Doljanski *et al.*, 1944; Doljanski and Werner, 1945).

Although biological media were sufficient for cultivation of cells their composition was unknown and

Table 1. Time table of first applications of antibiotics in cell culture

Year	Antibiotic	Applied concentration	Toxic concentration[a]	Tested cell types in culture	Investigator(s)
1940	penicillin	x		chicken heart cells leukocytes	Medawar (1940)
1941	penicillin	x			Abraham (1941)
1952	penicillin	<1000 U ml^{-1}	>1000 U ml^{-1}	human skin cells	Chruickshank and Lowbury (1952)
1945	streptomycin				Heilmann (1945)
1950	streptomycin	100–1000 U ml^{-1}	>1000 U ml^{-1}	embryonal cells	Ikegaki (1950)
1952	streptomycin	<1000 U ml^{-1}	>1000 U ml^{-1}	human skin cells	Chruickshank and Lowbury (1952)
1952	neomycin	<1 mg ml^{-1}		human skin cells	Chruickshank and Lowbury (1952)
1955	nystatin	<30 U ml^{-1} (=99 μg)	>250 U ml^{-1}	mammal cells	McLimans (1955)
1957	nystatin	<100 μg ml^{-1}	>200 μg ml^{-1}	mammal cells	Hemphill *et al.* (1957)
1957	amphotericin B	<40 μg ml^{-1}	>80 μg ml^{-1}	mammal cells	Hemphill *et al.* (1957)
1959	amphotericin B	1 μg ml^{-1}		mammal cells	Perlman (1959)
1959	fungizone	2.5 μg ml^{-1}	25 μg ml^{-1}	mammal cells	Perlman (1961)
1961	kanamycin				Smith (1959)
1967	gentamycin	50 μg ml^{-1}	>6 mg ml^{-1}	mammal cells	Casemore (1967)

[a] Toxic means: reduced cell growth, cell degeneration and cell death.

variable. As it was impossible to achieve reproducable results, there were numerous attempts to develop defined culture media.

The culture media were composed of metabolic products which were known to be essential for cell survival and development (Vogelaar and Erlichman, 1933; Earle, 1934; Baker and Ebeling, 1939; Fischer, 1939, 1941; White, 1946).

Later cell culture conditions were standardized by using defined media in order to achieve reproducable results. The first use of defined synthetic media without serum and without antibiotics was reported by Fischer and coworkers (1948), White (1949), Morgan and coworkers (1950) and Krebs (1950). Under such conditions mammalian cells could be cultured for up to several weeks, but not for prolonged cultivation periods. For prolonged cultures serum was used as a supplement in defined media. Some of these studies were done with, others without, antibiotics whereby the defined and therefore comparable conditions were partly annulled.

For example, investigations on cell growth were performed without antibiotics (Yasumura *et al.*, 1966; Choi and Bloom, 1970; Kano-Sueoka *et al.*, 1979; McKeehan *et al.*, 1987; Hoshi *et al.*, 1988) or with antibiotics (Owens *et al.*, 1954; Puck *et al.*, 1956; McLimans, 1957; Schindler *et al.*, 1959; Eagle, 1959, 1960; Tsao, 1982; Mckeehan and Adams, 1986).

Investigations concerning cell differentiation were also carried out without antibiotics (Sato, 1956; Yaffe, 1968; Augusti-Tocco and Sato, 1969; Sirbasku, 1978) or with antibiotics (Cahn R.B. and M.B. Cahn, 1966; Coon, 1966; Thompson *et al.*, 1966; Misfeldt and Sanders, 1987; Chung *et al.*, 1982; Lynch and Balaban, 1987; Dickmann and Mandel, 1989).

The standardization of cell culture conditions was mainly developed by Barnes and Sato. Attempts were made to replace the serum containing media with serum-free culture media, as sera show great variability in its composition. Both scientists developed serum-free media for many cell lines and worked without antibiotics (for summary: Barnes and Sato, 1980). Other investigators who prepared serum-free media also worked without antibiotics (Hayashi and Sato 1976; Bettger *et al.*, 1981; McKeehan *et al.*, 1976, 1984; Hammond *et al.*, 1984).

Today the use of serum-free media or defined serum-substitutes has increased in order to standardize and to achieve comparable results. On the other hand the different and unstandardizable use of antibiotics partly annules the comparability of results (see section: Final Remarks).

Application of antibiotics in cell culture

Since the initial applications of antibiotics in cell culture, experiments have been performed with and without antibiotics. However, the prophylactic use of

Table 2. Use of antibiotics in 243 publications with cell cultures in three special journals (Exp. Cell Research, J. Cell Science, In Vitro) of the year 1992

Number	Penicillin	Streptomycin[a]	Gentamycin	Fungizione amphotericin B	Others[b]
1	10^4 U	10 mg	2 μg		
1	500 U	100 μg	400 μg		
1	400 U	50 μg			
1	250 U	250 μg			
1	200 U	200 μg	5 μg		
1	200 U	200 μg			
1	150 U	150 μg			
1	120 μg	100 μg			
1	120 μg	63 μg			
1	120 μg	50 μg			
1	110 U	110 μg			
1	100 U	50 g			
1	100 U	10 mg			
1	100 U	200 μg		0.5 μg	
1	100 U	200 μg			
66	100 U	100 μg			
5	100 μg	100 μg			
2	100 U	100 μg	50 μg		
1	100 U	100 μg	50 μg	250 μg	
1	100 U	100 μg	40 μg	2.5 μg	
2	100 U	100 μg		250 μg	
2	100 U	100 μg		25 μg	
2	100 U	100 μg		2.5 μg	
1	100 U	100 μg		5 μg	50 μg (1)
3	100 U	100 μg		0.25 μg	
1	100 U	100 μg		0.25 μg	1% (2)
1	100 U	100 μg		1 μg	
2	100 U	100 μg		1%	
1	100 U	50 μg		2.5 μg	
1	100 U	60 μg			
2	100 U	50 μg			
1	100 U	100 μg		or	100 μg (0)
1	100 U	25 μg			
1	100 U	10 μg			
1	100 U	100 ng			
1	100 U				
1	90 U	90 μg			
1	80 U	80 μg			
1	60 μg	100 μg			
1	50 μg		50 mg		
20	50 U	50 μg			
1	50 U	50 μg		25 μg	
2	50 μg	50 μg			
1	10 U	100 μg			
1	10 U	10 μg			

Table 2. Continued.

Number	Penicillin	Streptomycin[a]	Gentamycin	Fungizione amphotericin B	Others[b]
1	8 U	8 μg		0.6 μg	
1	5 μg	5 μg			
1	0.6 μg	0.5 μg			
1	0.5 U			1%	
1	10%		10%		
2	1%	1%			
1	24 μl			1.5 μg	
15	x	x			
3	x	x		x	
2	x	x			x (3)
1	x	x			x (1)
1	x	x			x (2)
2			x		
1				x	
1			16 mg		
1			200 μg	2.5 mg	
1			200 μg		
1			100 μg	100 U	
2			100 μg		
19			50 μg		
1			50 μg	0.25 μg	
1			50 μg		
1			50 μg	2.5 μg	1 mg (4)
1			50 μg		50 U (0)
1			40 μg	0.25 μg	
1			32 μg	2.5 μg	
1			10 μg	2.5 μg	
1			5 μg		
1			1 μg	0.25 μg	
1			50 ng		
1			1%		
1				30 μg	
1				0.5 μg	60 μg (1)
2					60 μg (1)
1					25 U (1), 50 μg (3)
1					50 μg (1)
20	antibiotica				
1	antibiotica		50 μg		
4	antibiotica-antimycotica-solution				
1	antibiotica-antimycotica-solution				0.5% (0)

[a] Since with streptomycin 100 U correspond to 100 μg, all U-declarations were changed into μg.
[b] (0) – nystatin; (1) – kanamycin; (2) – anti PPLO; (3) – neomycin; (4) – cefotaxim.
All declarations refer to 1 ml medium.

antibiotics was and still is not uniform (Table 2).

The most commonly used antibiotic is a combination of penicillin/streptomycin. Other common antibiotics are gentamycin, kanamycin, neomycin, amphotericin B and nystatin. All antibiotics are added to medium in various concentrations.

Table 2 shows a study of 678 publications from three scientific journals (Experimental Cell Research,

Journal of Cell Science, In Vitro). 460 out of 678 publications (67.8%) describe studies, which are conducted with cell cultures from vertebrates. Of these, 190 authors (41.3%) worked without and 243 (52.8%) worked with antibiotic prophylasis. In seven publications, no data regarding the addition of antibiotics, was provided. In 26 papers the information available refers only to 'antibiotics' or 'antibiotic-antimycotic-solution'. In 27 publications (5.9%) no information concerning methods was made available.

As described in Table 2, penicillin was added to media in concentrations ranging from 0.5 U ml^{-1} to 10 000 U ml^{-1} or from 1% to 10%, respectively. Streptomycin concentrations ranged from 0.5 μg ml^{-1} to 10 mg ml^{-1} medium. Gentamycin was used in concentrations ranging from 1 μg ml^{-1} to 16 mg ml^{-1}, or from 1% to 10%, respectively. Amphotericin B or fungizone were used from 0.25 up to 250 μg ml^{-1} medium. Nystatin was used at concentrations of 0.5%, 25 U ml^{-1}, 50 U ml^{-1} and 100 μg ml^{-1}, kanamycin at 50 μg ml^{-1} and 60 μg ml^{-1} and neomycin was used at 50 μg ml^{-1}. Additionally, the number of different antibiotic combinations was very high. Of the 243 cell culture studies in which antibiotics were added to the medium, 85 different combinations were reported. Only three antibiotic formulas were frequently used:

- 66 × penicillin/streptomycin, 100 U/100 μg ml^{-1} medium,
- 20 × penicillin/streptomycin, 50 U/50 μg ml^{-1} medium and
- 19 × gentamycin, 50 μg ml^{-1} medium.

Effects of antibiotics on cells in culture

To determine the effects of antibiotics on cells in culture one has to consider the mode of action of antibiotics as being bactericidal (penicillin, streptomycin, kanamycin, gentamycin) or fungicidal (amphotericin B, fungizone, nystatin) (Table 3).

The effects of antibiotics on proliferation and metabolism of cells in culture mainly depend on *pH*, the *protein content* of the media and on the *temperature stability* of the antibiotic. The efficiency of antibiotics is optimal at *pH* 7.5 to 8.0. The *protein content* mainly depends on the amount of serum used in the culture medium. It was found that the activity of penicillin was reduced by binding to serum albumin (Chow and McKee, 1945; Tompsett *et al.*, 1947). Antibiotics, especially different types of penicillin, vary in their *thermal stability*. Warming up antibiotics to 37 °C frequently reduces their activity significantly.

Table 3. Mode of action of the most commonly used antibiotics in cell culture

1.	Inhibitors of synthesis of cell wall	
	a) β-lactam antibioticum	– penicillin
2.	Inhibitors of functions of cell membrane	
	a) polyene makrolide	– amphotericin B
		– fungizone
		– nystatin
3.	Inhibitors of protein synthesis	
	a) aminoglycoside	– streptomycin
		– neomycin
		– kanamycin
		– gentamycin
	b) makrolide	– tylosin
	c) different	– tiamulin
4.	Inhibitors of DNA synthesis	
	a) quinolone	– ciprofloxacin

Investigations in cell culture systems have revealed a reduced activity of penicillin which depends not only on the amount of serum but also on the species of the serum donor. Rolison and Sutherland (1965) demonstrated a binding capacity of penicillin to horse serum of 41%, and to calf serum of 37%. Lange and Assmann (1966) detected a binding capacity of penicillin to calf serum of 58% (±4%). Furthermore, the serum binding capacity differs between 30% and 93.5% between different penicillins (Rolison and Sutherland, 1965; Schafer *et al.*, 1972).

Although the activity of penicillin is directly influenced by pH, it is also indirectly influenced by the pH dependent binding of proteins. Protein binding is reversible (Keen, 1966; Barza *et al.*, 1972). The aminoglycoside antibiotics (here: streptomycin, kanamycin, gentamycin) differ strongly in their binding capacity to serum proteins and therefore show large differences in activity and period of activity (Paul and Beswick, 1982). The results for streptomycin range from 30% to 35%, whereas for kanamycin and gentamycin no serum binding was demonstrated (Gordon *et al.*, 1972).

The binding of antibiotics to serum proteins depends not only on the pH, but also on the concentration of magnesium and calcium in the culture medium. This effect was described for gentamycin (Ramirez-Ronda *et al.*, 1975; Gilbert *et al.*, 1971). Since each medium differs in its concentration of calcium and magnesium, so will the activity of gentamycin differ with each media.

None of the antibiotics used prophylactically in cell culture are described as affecting *DNA synthesis* .

Banck and Forsgren (1979) described this phenomena for penicillin and aminoglycosides. Neftel and coworkers (1989) however reported an influence on DNA-replication.

Protein biosynthesis is hindered by aminoglycosides (streptomycin, gentamycin, kanamycin, neomycin).

The polyene macrolides amphotericin B, fungizone and nystatin affect the *function of the cell membrane* since they are fungistatics and therefore affect the permeability of the membrane to various substances. Penicillin inhibits bacterial membrane synthesis. It also affects mammalian cells, as described below.

These statements indicate that results achieved in cell culture differ according to antibiotics used although identical conditions are assumed. Initial studies concerning the application of antibiotics in cell culture conditions focussed on the effectiveness of antibiotics on bacteria and fungi as well as their direct effect to cells. Later, more exact investigations were conducted using more sensitive methods.

– Effects of the beta-lactam antibiotic penicillin

The anti-bacterial effect of penicillin is caused by its interaction with special enzymes located in the cell wall of bacteria. Eukaryotic cells lack such structures. For this reason it was concluded that eukaryotic cells are insensitive to penicillin and its toxic effect. Further investigations revealed that the 'relatively low toxicity' of penicillin to mammalian cells is due to low permeability of cells to penicillin rather than low reactivity with the cell membrane (Eagle, 1954). Alternatively, Lange and Assmann (1966) demonstrated, that depending on the amount of serum in the media, penicillin is able to penetrate eukaryotic cells either by binding to proteins or by adsorbance by the cell surface. At 58%, the intercellular penicillin concentration reaches a maximum and begins to decrease, most likely due to degradation. The authors pointed out that serum causes reduction in antibiotic activity.

Studies on the toxicity of penicillin showed that cell vitality and enzyme leakage in rat liver cells cannot be influenced by the application of 100 μg ml^{-1} penicillin. In contrast, the incorporation of tritiated valine in intracellular proteins is reduced by up to 23%. This can either be due to a reduction of protein biosynthesis, to protein degradation or to a combination of both (Vonen and Morland, 1982).

Schwartze and Seglen (1981) demonstrated a 10% reduction of protein synthesis with the same concentration of penicillin. These findings indicate that penicillin affects the protein metabolism of liver cells. A concentration-dependent, antiproliferative effect of penicillin has also been demonstrated for other eukaryotic cells such as human bone marrow cells (Neftel *et al.*, 1985, 1986), murine lymphoma and cytotoxic cells (Huegin *et al.*, 1986) and human erythroleukemia cells (Cottagnoud and Neftel, 1986).

Studies on *in vitro* immunization showed that antigen specific proliferation of lymphocytes is inhibited by penicillin. This antiproliferative effect of penicillin is increased by degradation- or transformation by products of penicillin (Neftel *et al.*, 1983; Huegin *et al.*, 1986; Neftel and Müller, 1986; Neftel and Hübscher, 1987). Further studies revealed a common mechanism for the antiproliferative effect of all beta-lactam antibiotics. Therefore, DNA-replication is considered to be a primary target of these antibiotics (Fram, 1986; Cottagnoud and Neftel, 1986; Hübscher *et al.*, 1986, Huynh *et al.*, 1987).

In studies concerning the effect of cytostatics on cells in culture, interactions with beta-lactam antibiotics have to be considered. Altogether the effects of penicillin on eukaryotic cells raise the possibility that beta-lactam antibiotics are the cause of artefacts in cell culture investigations (Neftel and Hübscher, 1987).

– Effect of polyene macrolides (amphotericin B, fungizone, nystatin)

The first studies which examined the toxicity of the antimycotics nystatin and amphotericin B in tissue culture demonstrated that these substances are not toxic when used in proper concentrations (McLimans *et al.*, 1955, 1956; Hemphill *et al.*, 1957; Perlman *et al.*, 1959). Nystatin was found to be toxic to HeLa cells at concentrations exceeding 250 U ml^{-1} (82.5 μg ml^{-1}) and to chicken fibroblasts at concentrations greater than 500 U ml^{-1} (165 μg ml^{-1}) (McLimans *et al.*, 1955, 1956). Other authors described amphotericin B as toxic at 80 μg ml^{-1}, nystatin at 200 μg ml^{-1} (Hemphill *et al.*, 1957) and fungizone at 25 μg ml^{-1} (Perlman *et al.*, 1961).

The criteria for the toxic effect was cell death or cell degeneration. These rough parameters however, do not include all of the other consequences to the cell. McLimans (1955) observed that the missing evidence for the toxic effect could be caused by low sensitivity of available methods.

Therefore, it should be possible to detect effects of antimycotics on cells with more precise methods.

Polyene macrolides disturb the function of cell membranes and therefore their permeability. Thus a loss of substrates or enzymes is to be expected. In muscle cells, 43% to 90% of creatin-phosphokinase (CPK) is lost depending on the added amphotericin B concentrations (50 μg ml^{-1} to 500 μg ml^{-1}). This CPK leakage was accompanied by swelling of the nucleus, the endoplasmatic reticulum and the mitochondria (Laska *et al.*, 1990).

These results clearly contradict the initially described harmlessness of antimycotics in cell culture.

Effects of aminoglycosides (here: streptomycin, kanamycin, gentamycin)

The first studies which examined the toxicity of *streptomycin* in cell culture used the absence of cell growth as a measure of toxicity. Examination of the effects of streptomycin on mitosis at concentrations exceeding 250 U ml^{-1}, revealed that chicken embryo cells showed pycnotic chromosome changes during metaphase (Keilova, 1948). Streptomycin hinders amino acid transfer by binding to the 30 S ribosomal subunit and leads to errors in sequence transcription and to decreased protein synthesis (Collins, 1965; Hartmann *et al.*, 1968). Reduction of protein synthesis has also been demonstrated in cultured chicken muscle cells even at streptomycin concentrations as low as 25 μg ml^{-1}. Moreover, streptomycin (100 μg ml^{-1}) delayed the striations of myotubes (Moss *et al.*, 1984).

Like other aminoglycosides, streptomycin is nephrotoxic. Therefore, the effect on renal tubule cells is of special interest. Effects of streptomycin (100 μg ml^{-1}) on flounder renal proximal tubule cells include an abolished transepithelial potential difference, a decrease in intercellular glucose transport and lactate production and a reduced number of apical microvilli and large lysosomal stuctures (Dickmann and Renfro, 1990). Sens and coworkers (1988) found similar results in cultured human renal tubule cells. Further investigations described these 'myeloid-bodies' as an accumulation of phospholipids in lysosomes (Ghosh and Chatterjee, 1987; Ramsammy *et al.*, 1989).

Gentamycin was established in 1967 by Casemore for use in cell culture. At a concentration of 50 μg ml^{-1}, it was found to be very effective against bacteria and hardly toxic to cells. At 50 μg ml^{-1}, no morphological changes were observed microscopically and no deviations in growth behaviour were noticed (Schafer *et al.*, 1972). However, massive morphological changes became obvious in embryonal chicken fibroblasts, mouse fibroblasts and HeLa cells when 6 mg ml^{-1} gentamycin was added to culture media (Schafer *et al.*, 1972). Viano and coworkers (1983) demonstrated dosage-dependent toxicity to rabbit renal cells with a gentamycin concentration ranging from 0.5 mg ml^{-1} to 4 mg ml^{-1}. Toxicity was determined by cell death as measured by neutral red uptake and the release of lactate dehydrogenase. In contrast, toxicity to human diploid lung fibroblasts was observed at gentamycin concentrations of 100 μg ml^{-1}. Here, cell adhesion to tissue culture flasks was used as a measure of toxicity. The toxic effect of gentamycin is explained by a distinct accumulation of this antibiotic in cellular lysosomes (Litwin, 1970). Tulkens and Trouet (1974) demonstrated an accumulation of gentamycin and kanamycin in embryonal rat fibroblasts. Aubert-Tulkens and coworkers (1979) showed that at a concentration of 250 μg ml^{-1}, gentamycin enlarges the cell volume and reduces the number of cellular lysosomes.

Increased cell volume occurs as a result of an accumulation of phospholipids. This accumulation is explained by an obvious reduction in the activity of lysosomal sphingomyelinase.

Hori and coworkers (1984) discovered a decrease in activity of another lysosomal enzyme, n-acetylglucosaminidase, after gentamycin treatment. They also demonstrated a decrease in intracellular cAMP. An increase in lysosomal enzyme concentration in the medium occurs as a result of increased cell permeability, after gentamycin addition.

A reduction of ATP concentration in the mitochondria of renal cells, which leads to an inhibition of oxidative phosphorylation, was demonstrated by Simmons and coworkers (1980). Furthermore the lactic acid concentration in the medium of gentamycin treated swine renal cells is reduced. In addition, the turnover rate of mono- and diglycerides is increased, whereas that of triglycerides is decreased (Schwertz *et al.*, 1986).

The above list of morphological and functional changes in cells after addition of gentamycin to culture medium, are dosage dependent. They can be observed at gentamycin concentrations which are commonly used in cell culture. Moreover, they are dependent on cell type and on the species of the cell donor. These changes are considerable and influence the results of cell culture investigations.

Prophylactic precautions for the sterile handling of cell cultures

Prophylactic addition of antibiotics to culture media is a widespread method of 'sterile working' in cell culture research. As described above, antibiotics have many effects on cells.

In order to yield reproducable results, antibiotic-free cell culture conditions are necessary. Germ-free cell culture working conditions refer to those conditions which reduce the probability of contamination as much as possible, since absolute sterility is not possible. To achieve this, accurate techniques for sterile cell culture work is necessary.

Final remarks

The present work describes the effects of the most commonly used antibiotics on cell cultures. These effects are dependent on the serum concentration used in the media and on the species of the cell donor. The influence of antibiotics on cell cultures varies between different species, organs and cell lines, because of their differences in susceptibility to the effects of antibiotics. Statements on the general toxicity of antibiotics are always influenced by the above mentioned parameters.

In addition the term toxicity is not clearly defined. In early cell culture studies, cell growth, degeneration and death were the main measures of toxicity. Later definitions described toxicity as a measure of the effect of antibiotics on cell metabolism. Moreover, concentrations of antibiotics in cell culture considered to be 'non toxic' may in fact disturb cellular functions.

In 1979, Perlmann stipulated that for antibiotics to be prophylactically used in cell culture, these antibiotics must not interfere with cell growth and metabolism. As demonstrated in the present paper these demands are difficult to fulfil. As seen in Table 2, the concentrations of antibiotics used in cell culture medium vary considerably. While some very high dosages (10^4 U ml^{-1}, 50 μg ml^{-1}, 50 mg ml^{-1}, 16 mg ml^{-1}, 10 mg ml^{-1}) are unreasonable since these concentrations cannot be used in medium, other dosages (200–500 U ml^{-1} penicillin, 200–250 μg ml^{-1} streptomycin, 100–400 μg ml^{-1} gentamycin, 25–250 μg ml^{-1} and 100 U ml^{-1} fungizone or amphotericin B) are high and clearly above a toxic concentration. The results of these investigations have to be seriously questioned.

In addition some antibiotic doses are clearly below the effective concentration (0.6–5 μg ml^{-1} and 0.5–10 U ml^{-1} penicillin, 0.5–25 μg ml^{-1} streptomycin, 50 ng–10 μg ml^{-1} gentamycin, 0.25–1 μg ml^{-1} fungizone). Antibiotics should never be underdosed since their antimicrobial activity is not effective and may lead to the establishment of resistent germs. Lindl and Bauer (1989) observed that the prophylactic use of antibiotics may cause workers to neglect sterile handling of cell cultures and therefore increase the possibility of development of resistant germs which lead to uncontrollable contaminations.

Contaminated hybridoma cell cultures are often sterilized by an 'animal passage'. This technique of decontamination of hybridoma cells causes pain, suffering and injury to the animal, because it leads to an infiltrative tumor growth in the abdomen (Kuhlmann *et al.*, 1989; Ruhdel, 1992).

In contrast, the *therapeutic* use of antibiotics in important cell lines and in primary cell cultures may be indicated as a hygienic tool. Antibiotics should be applied as recommended by the manufacturer. The antibiotic should be added to cell culture medium for approximately two weeks. During this time, the antibiotic-containing medium should be changed every two to three days because of the instability of the antibiotics. Studies with the cell lines should only be conducted once the antibiotics have been removed from the culture medium and the cells have been tested for sterility.

Results achieved under defined cell culture conditions are standardized. Therefore it has been the aim of many scientists to define exact parameters for cell culture conditions (for survey: McKeehan *et al.*, 1990).

The prophylactic use of antibiotics in cell culture medium is unsuitable. Antibiotics interfere with cells in culture, resulting in incomparable culture conditions and questionable results.

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