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Polar Application of Test Substances in an Organotypic Environment and under Continuous Medium Flow: A New Tissue-based Test Concept for a Broad Range of Applications in Pharmacotoxicology

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Abstract—We have established a new test concept for *in vitro* pharmacological trials. Our model employs tissue explants to test compounds for toxicity which arises with the metabolic interactions among different cell types. Microsurgical preparation of tissue explants avoids the destruction of the organ-specific tissue architecture. Explants were mounted in tissue carriers to improve nutrition and handling of the sample. To allow for the omission of serum supplementation of the culture medium, explants were cultured under continuous medium flow. Test substances are applied considering the polar architecture of most tissues *in vivo*, for example, covering the apical aspect of epithelia. In principal, all tissues obtained from any species, including man, can be used in this system. A trial application was performed with vitreous body substitutes, substances used in ophthalmology. One compound had passed cell culture tests, but caused massive blood vessel deterioration *in vivo*. Using our test system based on the developing renal vessel system, we could confirm, within 24 hours, severe vessel damage which resembles the injury suffered by the rabbit retina. We demonstrate that an improved tissue culture assay is a suitable tool for the detection of toxicity that remained unidentified in cell culture tests. © *2000 Elsevier Science Ltd. All rights reserved*

Keywords: toxicology; biocompatibility; *in vitro* test model; serum-free perfusion culture.

Abbreviations: IMDM = Iscove's modified Dulbecco's medium; PBST = phosphate buffered saline with supplements.

INTRODUCTION

During the legal procedures to introduce new drugs and cosmetics into the market, novel substances are routinely subjected to elaborate trials to prove their safety for the use in humans. Initial tests are conducted using cell-free test systems (Clemedson *et al.*, 1998), cell cultures (Babich and Borenfreund 1991; Barile 1997; Schmalix *et al.*, 1996) and microorganisms (Mitchell and Combes 1997; Purves *et al.*, 1995), followed by trials in animals such as mice, rats and rabbits, before their performance is finally evaluated in clinical studies on humans. The detailed verification process serves to assure the consumer that new substances are potent and do not cause adverse side-effects. Therefore, the development of reliable, safe and simple evaluation procedures for substances of medical or cosmetic use is of major concern, which has to address both economic and ethical questions.

Cell cultures have gained a long-standing recognition as the initial approach within drug verification procedures (for reviews, see Barile 1997; Wiebel *et al.*, 1997). However, experience has also shown that new agents, when tested in cell cultures, may not show any toxicity, whereas following animal trials reveal unexpected, severe side-effects

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(Ekwall, 1980, 1983). Such contradictory results demand attention since they demonstrate the need for additional procedures.

Animal and human organisms consist of a multitude of diverse cell types, interrelated by complex metabolic and signalling processes. It is an accepted fact that a substance itself may not be toxic, but its metabolites may exert toxic effects. Cell culture test systems usually consist of a single cell type. Thus, the uptake, effect and degradation behaviour of a drug in cell cultures do not necessarily resemble the responses when this drug is administered to tissues, organs or whole organisms (Bach *et al.*, 1996). Moreover, a drug that appears non-toxic to one cell type can be detrimental for others. However, the Multicenter Evaluation of In vitro Cytotoxicity (MEIC) programme impressively underlined the potential of *in vitro* test systems (Clemedson *et al.*, 1998). The restrictions of one single cell test system can be overcome by combining different cell test models or the combination with suitable tissue culture set ups.

In consequence, it is useful to determine the extent to which tissue culture approaches can overcome the mentioned restrictions (Bach *et al.*, 1996; Hawksworth *et al.*, 1995; Klug *et al.*, 1998; Spielmann *et al.*, 1996). These test systems rely on the natural tissue composition, the metabolic relations between the contained cell types, and on a normal state of differentiation. Thus, the test substances and their metabolites are evaluated within the framework of, and the metabolic interactions among, populations of different cell types. Moreover, tissue culture models also allow for the use of human tissue for toxicity testing *in vitro*. This would enable researchers, for the first time, to determine the effects of test substances on human tissue before launching a clinical study.

A key question in tissue culture remains whether tissue explants can be kept in culture while maintaining their organotypic state and functions. Perfusion tissue culture systems that provide organotypic culture conditions have been used for years and with a variety of tissues (Kloth *et al.*, 1995, 1998; Kobuch *et al.*, 1997; Minuth *et al.*, 1992, 1998; Sittinger *et al.*, 1994). The results of our previous studies on the developing kidney led to the question, and encouraged us to examine, whether the good preservation of tissue cultured under medium perfusion may be employed to establish a suitable *in vitro* toxicity test system.

Two approaches were pursued to test this concept. First we examined, whether the complex cellular compositions of kidney tissue and the expression of a cell specific antigen are in fact retained under perfusion culture. Secondly, we challenged an exemplary tissue culture model with a known test substance that had not shown any toxicity in cell cultures, but showed severe side-effects in animal experiments.

MATERIALS AND METHODS

Tissue preparation

1–3-day-old New Zealand rabbits were killed by cervical dislocation. Kidney and stomach were removed under sterile conditions and tissue samples of these organs microsurgically excised without protease treatment, thus retaining the organotypic cellular composition (Minuth, 1987). The nephrogenic zone of the kidney was prepared by stripping off the fibrous organ capsule using fine forceps (Dumont No. 5, Aesculap, Tuttlingen, Germany). The developing tissue zone (thickness 150–200 μm) remained attached to the fibrous capsule that served as natural support material.

The isolated samples were mounted in tissue carriers (Plate 1; Minucells and Minutissue, Bad Abbach, Germany). This counteracts the tendency of the specimens to curl due to unequal tissue tension. Mounting the samples also ensures constant conditions for nutrient and metabolite exchange during culture, and facilitates transfer to different culture chambers, or for analysis, such as microscopic examination. Artificial support material was not used in these experiments.

The mounted specimens were cultured under continuous medium exchange using perfusion chambers (Minuth *et al.*, 1992; Minucells and Minutissue, Bad Abbach, Germany). This technique allowed for the complete omission of serum or tissue extract supplements (Kloth *et al.*, 1994) in the culture medium, Iscove's modified Dulbecco's medium (IMDM; Gibco-BRL Life Technologies, Eggenstein, Germany). To stimulate vascular development, kidney explants were cultured for 13 days in IMDM, supplemented with aldosterone (1×10^{-7} M) and 1,25-dihydroxyvitamin D_3 (1×10^{-9} M). All experiments described herein were carried out at least in triplicate.

Comparative study of culture conditions

To determine the effect of perfusion on tissue preservation, parallel samples of kidney tissue were mounted as described and cultured under both stationary conditions and continuous medium perfusion (Plate 2). Stationary cultures were maintained in 24-well plates (Greiner, Frickenhausen, Germany) in an incubator at 37°C and a controlled atmosphere (95% air, 5% CO_2, 100% relative humidity). Medium was exchanged daily. Samples loaded in perfusion chambers were kept at 37°C and were perfused with 1 ml culture medium/hr as described above, prewarmed to the same temperature.

Toxicity test of vitreous body substitutes

Test procedures in vivo. To evaluate the intraocular long-term tolerance of perfluorcarbonliquids as artificial vitreous body substitutes, two different substances, perfluorophenanthrene (Vitreon®,

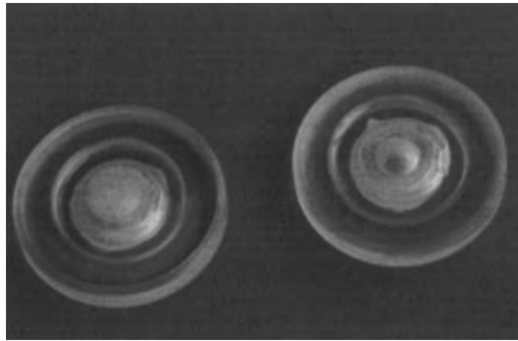

Plate 1. Tissue carriers for mounting of explants. Microsurgically excised sheets of tissue were placed in the centre of tissue carriers and mounted with flexible holding rings. This procedure eliminated curling of the sample due to tissue tension, and prevented damage when handling the explants. The tissue carriers fit in a variety of culture chamber designs (see Plates 2B and 5).

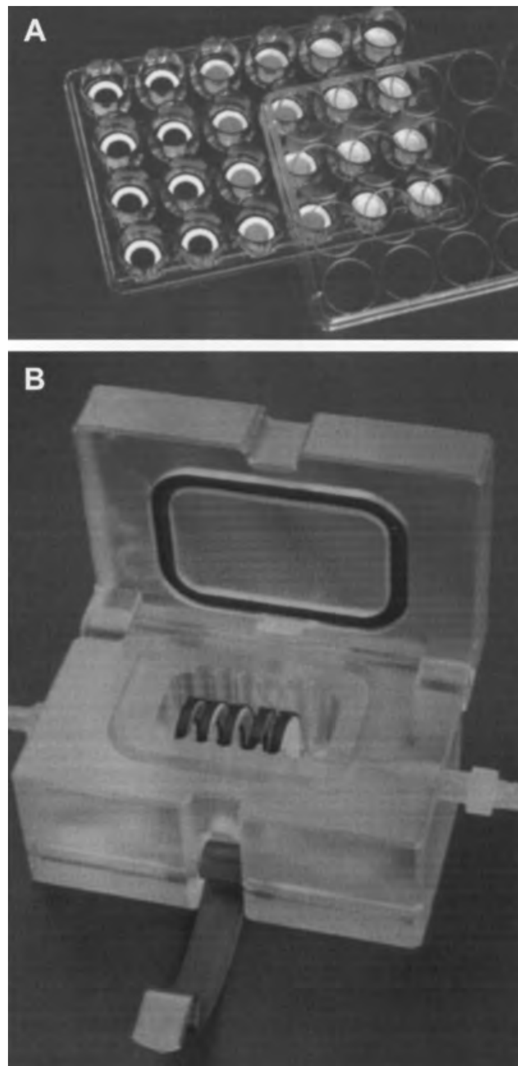

Plate 2. Stationary and perfusion culture. (A) For stationary culture, mounted tissue specimens were maintained in 24-well plates in a cell culture incubator and medium was replaced daily. (B) Perfusion chambers were loaded with multiple mounted tissue samples. The sealed chambers were maintained on a warming plate at 37°C and serum-free culture medium was constantly pumped (1 ml/hr) through the chamber.

Vitreophage Inc., Lyons, USA) and perfluorodecalin (Adato®deca, Chiron Adatomed GmbH, Dornach, Germany) were tested in one eye of four rabbits each.

Animal experiments were performed in accordance with the ARVO-statement for the use of animals in ophthalmic research. All surgical procedures were performed under general anaesthesia by intramuscular injection of 35 mg ketamine/kg (Ketavet, Parke-Davis, USA) and 2 mg xylazine/kg (Xylazine, Bayer, Germany). After gas-compression of the vitreous body, 1.2 ml vitreous body substitute (perfluorophenanthrene or perfluorodecaline, respectively) was injected into the vitreous cavity of the eye, so that two-thirds of the vitreous body was replaced by the test substance and that the area of the rabbit's retinal blood vessels was completely covered by the artificial substitute. Post-operatively, eyes were examined by indirect ophthalmoscopy, Schiötz-tonometry and video fluorescein angiography. After observation intervals of 2 and 6 wk the animals were sacrificed, and the eyes were processed for histological examination.

To investigate the preservation of the integrity of the blood vessels, the vascular bed of the retina was prepared. The eyes were cut into halves and were fixed in 10% formalin overnight. Flat specimens of the retinal vascular network were prepared according to the method described by Kuwabara and Cogan (1960). The retinae were incubated at 37°C for 3 hr in a 3% trypsin solution (1:250 porcine pancreas trypsin, Sigma, Deisenhofen, Germany, in 0.1 M Tris buffer, pH 7.8). Using this procedure, the surrounding retinal tissue is digested while the retinal vascular network is preserved. The specimens were stained by PAS-HE. The untreated contralateral eyes and two eyes after intravitreal injection of balanced salt solution were used as controls. The control specimen were treated as described above.

Test procedures in vitro. The tolerance of perfluorophenanthrene and perfluorodecaline *in vitro* was analysed using a kidney tissue culture model under perfusion conditions as described above. In accordance with the application in question, the compounds were administered using a gradient culture chamber (Plate 5; Minucells and Minutissue), which features separate upper and lower compartments that are separated by the specimen itself, mounted on the tissue carrier. After loading the sample, the upper compartment was filled with the test compound, whereas the lower compartment was perfused with medium. This approach allowed to statically apply the test compounds in one compartment while maintaining the necessary continuous medium exchange for the specimen through the other compartment. This experimental set-up mimics the *in vivo* test conditions where vitreous body substitutes are in unilateral contact with the test tissue. In control experiments, the upper chamber was stationary filled with culture medium. The gradient chambers were maintained at 37°C and perfused for 24 hr at 1 ml/hr. Tests were carried out in triplicate.

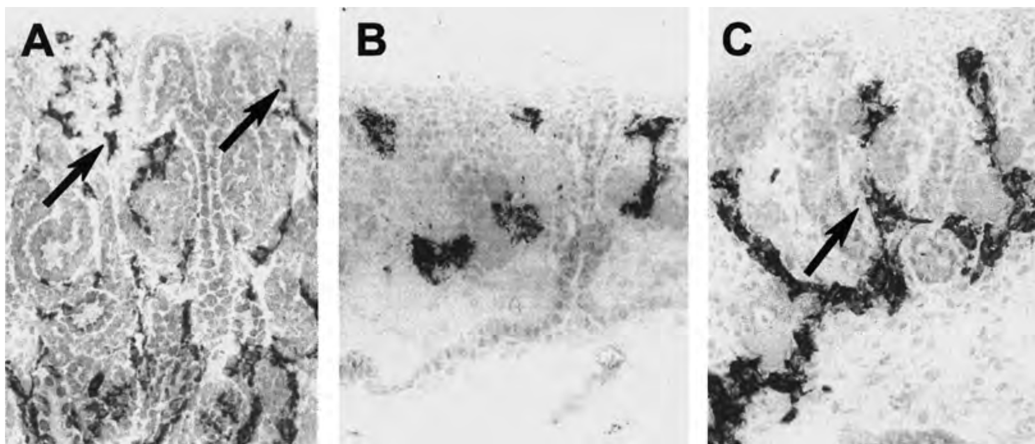

Plate 3. Structural preservation of neonatal rabbit kidney (A–C) tissue explants under serum-free stationary and perfusion culture. (A) Kidney—initial conditions before culture, longitudinal section of the cortex. The developing vascular network of the kidney is shown after immunostaining with the endothelium-specific antibody EC1. Vessels (arrow) protruding between the collecting duct ampullae towards the *capsula fibrosa* were clearly labelled, as were the vascular elements of developing glomeruli. (B) After 13 days of stationary culture, the organotypic organization of the tissue, as well as the regular arrangement of vessels, were widely disintegrated. Irregular patches of endothelial cells are dispersed within the tissue, which also featured numerous necrotic cells. (C) In contrast, explants perfused for 13 days showed good preservation of the organotypic composition. Cells with pycnotic nuclei were scarce. The vascular network had gained size and dimension, the endothelial cell streaks showed heavy immunolabelling (arrow). Magnification (A–C) × 175.

Immunohistochemistry

Expression of an endothelium-specific antigen was detected with the monoclonal antibody EC1 (Kloth *et al.*, 1994), following the procedures described therein. Cryosections (8 μm thickness) were fixed with ethanol, washed with phosphate buffered saline (PBS), blocked with PBS supplemented with 20% horse serum (Sigma, Deisenhofen, Germany) and 0.8% Triton X-100 (Pierce, Rockford, USA; PBST), and incubated with the specific antibody (supernatant diluted 1:2 in PBST) overnight. Specimens were then treated with species-specific biotin-antibody conjugates (Dianova, Hamburg, Germany; 1:300 in PBST). The specific binding was enhanced using streptavidine-conjugated horseradish peroxidase (Vector, Burlingame, USA), according to the manufacturer's protocol. To verify the specificity of the reactions, either mouse pre-immune serum, or non-specific primary antibody controls were included in each experiment. Sections were examined with a Zeiss Axiovert 35 light microscope and documented on Agfapan 25 film (Agfa, Leverkusen, Germany).

To avoid unintentional bias the analysis of the tissue sections was carried out using coded samples. The experimenter evaluating the histochemical specimens had in no case any information about the type of sample.

RESULTS

Good preservation of tissue morphology using perfusion culture

For our comparative study we used neonatal rabbit kidney. The elaborate developing vascular network of the kidney features an outstanding degree of spatial organization (Plate 3a; for a detailed survey see Kloth *et al.*, 1997) that makes it an ideal and sensitive marker structure to determine even minute signs of damage. Moreover, as a properly developed vasculature is an important prerequisite for normal organ function, pathological alterations of the vascular network caused by xenobiotics will result in serious damage to these functions, too.

Stationary culture with serum-free medium proved insufficient to maintain the typical cellular composition of the tissue. (Plate 3b). Within the culture period explants showed extensive necrotic alterations and high proportions of cells with pycnotic nuclei. The typical architecture of the nephrogenic zone was completely destroyed. Endothelial cells formed large clusters, blood vessels were never detected in these samples.

Perfusion culture, in contrast, yielded high specimen viability, retention of the cellular diversity and tissue composition. The vascular network of kidney explants (Plate 3c) continued to grow under these conditions, and showed no necrosis at all. Both the extension of existing vessels up to 300 μm and an increase in diameter were observed.

Trial application: toxicity test of vitreous body substitutes

Chemical substitutes are used in ophthalmology to replace a damaged or destroyed vitreous body (Chirila *et al.*, 1994). We examined two vitreous body substitute compounds, perfluorophenanthrene and perfluorodecaline, which had been identified as non-toxic in cell culture tests (Nabih *et al.*, 1989). Perfluorophenanthrene had caused serious pathologic changes of the retinal vascular system in the rabbit eye (Kobuch *et al.*, 1997), whereas perfluorodecaline had shown less severe side-effects in animal experiments. Both substances were tolerated in the rabbit eye without inflammatory reaction for the observed period of up to 6 wk. One side-effect was that the initially clear bubble of substance started to divide into droplets after a few days, which would hinder the vision of a patient considerably. However, the most obvious damage was observed in the retinal blood vessels (Plate 4). Routine fluorescein angiography of the retinal vessels demonstrated severe vascular deterioration (Plate 4a): narrowing of vessels, formation of microaneurysms, vascular occlusions, areas of vascular retraction and leakage of fluorescein from the vessels, which suggests major damage to the integrity of vessel walls. These serious alterations were present to a much higher degree after intravitreal application of perfluorophenanthrene than after perfluorodecaline. These clinical data were paralleled by histological observations. The trypsin-digested preparations of the retinal vascular system showed variations in the calibre of the vessels, blurred demarcation of the vessel walls, and multiple nodular and saccular aneurysms. In some areas, the complete loss of both perivascular and endothelial cells left behind "vessel ghosts" (Plate 4c), tubular matrix structures devoid of any cells. The morphological alterations of the vessels were observed after application with both substances, but were far more striking after perfluorophenanthrene treatment.

The primary experimental task of this study was to determine whether vascular damage caused by perfluorophenanthrene *in vivo* could be detected *in vitro* using a tissue culture model. As a test system we chose the neonatal rabbit kidney, since the high degree of vascular organization in this tissue may easily be affected by any substance that damages the vasculature. A gradient perfusion chamber was used for these experiments. The test substance was applied on one side of the explant, while the other side of the tissue was continuously supplied with fresh culture medium. This application form mimics the *in vivo* test conditions, where the vitreous body substitute performs unilateral contact with the underlying tissue and its vascular network.

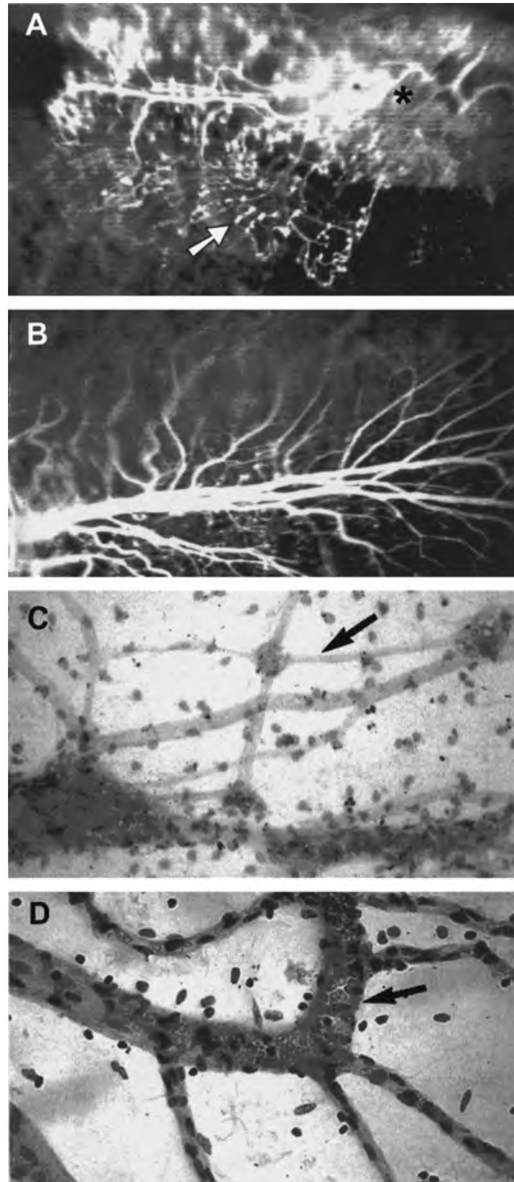

Plate 4. Toxicity test of vitreous body substitutes in the rabbit eye. (A and B): The most striking clinical findings were revealed by routine fluorescein-angiography of the retinal vessels. (A): 6 wk after perfluorophenanthrene injection narrowing of the blood vessels, microaneurysms (arrow), and leackage of fluorescein-dye from the vessels (asterisk) demonstrated vascular deterioration. (B): Vascular pattern of the normal untreated eye. (C and D): These clinical *in vivo* findings were confirmed by histological data of the retinal vessels. Sheets of retina prepared from treated rabbit eyes were trypsinized, which removes the cells but retains the vascular network. (C) Perfluorophenanthrene application resulted in severe damage to the vascular network. The remaining vascular structures were characterized by multiple nodular and saccular aneurysms. Perivascular cells were no longer detectable. Instead, cell-free tubular strands (''vessel ghosts'', arrow) were observed. (D) The retinal vascular network of the intact eye consisted of arterioles (arrow) featuring perivascular cells, and numerous capillaries. Magnification $\times$ 200.

Within 24 hr, samples exposed to perfluorophenanthrene had lost most of their vascular structures (Plate 6a). Blood vessels were absent in the most part of the explant, and remaining endothelial cells were arranged in isolated clusters. In this short time frame, explants exposed to perfluorodecaline showed no visible damage of the vascular network (Plate 6c,d), as did the untreated control specimens. Despite this deleterious effect of perfluorophenanthrene on the vessel structure, no correlation was observed when we examined the expression of the EC1-antigen using immunohistochemistry. The endothelium-specific marker was highly expressed in perfluorcarbon liquid-treated tissue and in the untreated controls (Plate 6). Blood vessel deterioration *in vitro* took place within a period as short

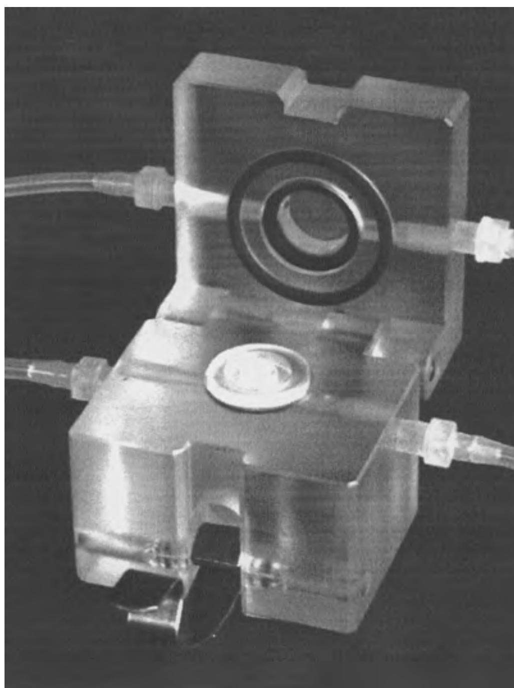

Plate 5. Gradient perfusion chamber. For toxicity testing of vitreous body substitutes, a gradient perfusion chamber was loaded with a single tissue sample. In this chamber design, the mounted sample itself creates the barrier between a lower compartment in the base, and an upper compartment in the lid, which allows for separate perfusion of the compartments. For the experiments described here, the upper compartment was filled, but not perfused, with the test substances, while the lower compartment was perfused with serum-free culture medium.

as 24 hr, thus the tissue culture test was able to verify *in vitro*, the main result of a long-term animal experiment.

DISCUSSION

The significance of perfusion culture for tissue preservation in vitro

How can the quality of tissue preservation under perfusion culture be explained? One major improvement achieved when using medium perfusion is the continuous supply with nutrients. The permanent replacement of the medium, however, has more benefits to offer. Metabolites, paracrine factors and secretory products are drained with the medium flow and thus do not accumulate to reach unphysiological concentrations in the culture chamber (Minuth *et al*., 1998).

The second main advantage of the tissue culture approach described here is the use of a culture medium that does not contain serum components (Kloth *et al*., 1994, 1995). Serum quality and composition, as well as growth and differentiation factor contents, differ greatly between batches, and serum also contains components with still unknown functions. Thus, the omission of serum constitutes a major improvement towards reproducible culture conditions.

As shown in the nephrogenic zone of the neonatal kidney, differentiation processes can persist during serum-free perfusion culture (Plate 3c). The morphology of complex tissues can be preserved, and functional aspects, for instance the secretory activity of specialised cell types, are maintained as well (Kloth *et al*., 1998).

Approaches and perspectives for toxicity testing using organotypic tissue culture models

The main goal of this study was to investigate whether an organotypic tissue culture model can be a reliable means for the sensitive detection of toxic effects of chemicals *in vitro*. It is a well-known fact that the response of the organism to drug administration depends on complex physiological interrelationship among the different cell types and organs of the body. Based on these considerations, a new *in vitro* test concept was established. Tissue explants are prepared without the application of proteases. Thus, the organotypic architecture of the tissue remains intact, which provides a fundamental prerequisite for organ-specific cellular interactions under *in vitro* conditions (Bach *et al*., 1996). Continuous perfusion of the back side of the tissue explant safeguards optimal nutrition and constant removal of metabolites (Minuth *et al*., 1998). The use of serum-free media in this culture model elim-

inates the influence of undefined serum components on the reproducibility of experiments. An additional new feature of our test concept is that liquid test substances can be applied unilaterally, which mimics common *in vivo* pathways of chemical exposition and drug application, for example, through epithelial surfaces.

For the investigations described here the developing vasculature of the kidney was employed. This tissue is characterized by an extraordinary high degree of spatial organization (Kloth *et al.*, 1997), which makes it an ideal candidate for the detection of minute toxic effects imposed on the blood vessel system. As an example application, we chose perfluorcarbon liquids, some of which account for severe vessel damage when applied in an animal test model over prolonged periods (Kobuch *et al.*, 1997). Preceding the *in vivo* animal experiment, both compounds used in this study had successfully undergone toxicity tests on standard cell culture lines where no adverse side effects could be detected (Nabih *et al.*, 1989). Reports on vascular effects of perfluorcarbon liquids in the eye are scarce so far (Kobuch *et al.*, 1997; Velikay *et al.*, 1993). Accordingly, the factors responsible for vascular reactions in perfluorcarbon liquid-treated eyes are mostly unknown and are subject to current discussion. Although most perfluorcarbon liquids have rather similar physical and chemical properties, biophysical differences between perfluorophenanthrene and perfluorodecalin could be responsible for their different toxicity. Perfluorophenanthrene possesses a higher specific gravity than perfluorodecalin (double that of normal vitreous). Nutrients and metabolites are almost insoluble in perfluorophenanthrene, and it has a very high O_2-binding capacity compared to the internal O_2-partial pressure of intact vitreous. Remarkably, hyperoxic conditions are known to stimulate the local regression of blood vessels (Pierce *et al.*, 1996). Vascular regression is preceded by the loss of perivascular cells (Benjamin *et al.*, 1998) since it was observed in the *in vivo* experiments. At present impurities of the substances are considered the main causative factor for toxic reactions (Meinert *et al.*, 1995, Velikay *et al.*, 1993). Free and bound hydrogen as well as C-double bond-containing by-products are known to be highly tissue-toxic and cannot be excluded completely in the manufacturing process. Thus, a sensitive *in vitro* test system will be a valuable tool for quality assessment of the manufacturing process. Furthermore, tissue culture models will help to elucidate the mechanisms underlying the blood vessel deterioration following application of distinct perfluorcarbon liquids.

Two parameters were used to determine the toxicity of perfluorcarbon liquids in the tissue culture test described above: (1) specific surface antigens detected with antibodies, and (2) damage to the highly ordered vascular network of the tissue explants. This spatial order is tied to a distinct state of differentiation, it reflects the co-ordinated development of several organ components (see Kloth *et al.*, 1997). Hence, the structural preservation of the three-dimensional vascular network as well as the integrity of developing blood vessels are sensible parameters for the detection of toxicity.

The application of perfluorophenanthrene resulted in severe deterioration of the developing blood vessels of the tissue explant. The expression of the EC1 antigen was not affected. Large EC1-labelled endothelial clusters were detected in perfluorophenanthrene-treated samples. However, the integrity of the vessels and the architecture of the vascular network was destroyed. None of these drastic changes was induced by perfluorodecalin or in the control experiment. Whether perfluorophenanthrene itself or by-products of the manufacturing process caused the destruction of the vessels remain to be examined. None the less, the tissue

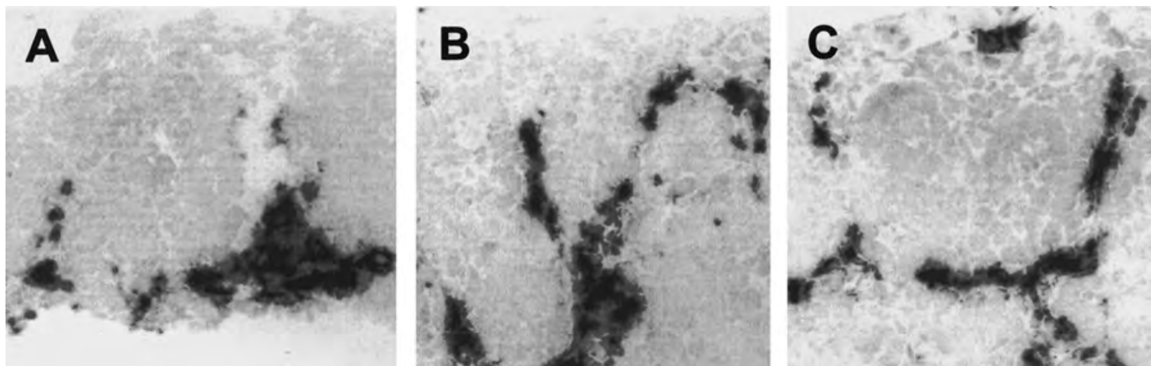

Plate 6. Toxicity test of vitreous body substitutes in the renal tissue culture model. (A) Within 24 hr of culture, perfluorophenanthrene had caused severe damage to the vascular network. Only isolated endothelial cells and a few cell clusters were detected. (B) Otherwise, adverse effects of perfluorodecalin were not observed, immunostaining revealed the characteristic patterning of endothelial cell strands. (C) Control experiment. Culture medium filled stationary in the upper compartment did not alter the normal patterning of the vascular network. Magnification × 350.

culture model sensitively detected the toxicity of this preparation that has been found to be non-toxic in a previous *in vitro* test based on a single cell type (Nabih *et al.*, 1989). These toxic effects were detectable already after 24 hr of application. The outcome of the *in vitro* tissue culture test is in excellent agreement with the results of the *in vivo* experiments. Both test set ups unequivocally revealed severe vascular damage.

Morphological criteria are only one set of parameters for toxicity testing applications. Although morphology can target highly sensitive mechanisms and interrelations within the tissue, the evaluation requires time and expertise. Unless the test calls, for instance, for the proof of successful drug delivery to a certain cell type, many applications call for different means of measurement. Tissue culture models are suitable platforms for on-line toxicity tests, where the amounts or properties of metabolites or secretory products in the culture supernatant are monitored and recorded in an automated fashion. As perfusion culture itself constitutes a flow-through system, it just depends on the right choice of parameter(s) and sensor(s) to develop significant, automated assays. The simplicity and modular design of the culture system facilitates experimental modifications. Medium flow rates, duration of experiments and culture medium composition can be tailored to meet individual demands.

Cell culture models have proven excellent tools to determine the acute toxicity of chemical compounds. However, if the toxic effect of a substance results from the interaction of different cell types, these models are bound to fail. Here, organotypic tissue culture models offer an innovative approach capable to provide new insights for *in vitro* toxicity testing. The inherent versatility of tissue culture models as described in this study bears novel opportunities to approach complex cellular interactions in drug testing, and thus has a huge potential for the improvement of *in vitro* test procedures.

A new test concept for *in vitro* pharmacological trials was established. It employs tissue explants that retain their organ-specific composition to test compounds for toxicity, which arises with the metabolic interactions among different cell types. In principal, all tissues obtained form any species, including man, can be used. Test substances are applied considering the polar architecture of most tissues *in vivo*, for example covering the apical aspect of epithelia. Medium perfusion accounts for good sample preservation and simplifies on-line measurement of parameters.

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