From the Departments of Infectious Diseases and Pharmacology, University of Umeå, Umeå, Sweden

**Neurotoxicity of β -lactam Antibiotics.
Experimental Kinetic and Neurophysiological Studies.**

AKADEMISK AVHANDLING

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Abstract

The neurotoxic potential of intravenous administered benzylpenicillin (BPC) was studied in rabbits with intact blood-CNS barriers and rabbits with experimental *E. coli* meningitis. At onset of epileptogenic EEG activity or seizures, serum, CSF and brain tissue were collected for assay of BPC. Based on the fact that, in tissues, BPC seems to remain extracellularly, brain concentrations of BPC were expressed as brain tissue fluid (BTF) levels, calculated as 10x the concentration in whole brain tissue. Neurotoxicity could be precipitated in all rabbits. In normal rabbits BTF levels of BPC were considerably higher than those in CSF indicating a better penetration across the blood-brain barrier (BBB). BPC penetrated better to CSF and BTF in meningitic rabbits than in normal controls, suggesting some degree of damage of the BBB concomitant with meningeal inflammation. *E. coli* meningitis did not increase the neurotoxicity of BPC. In control rabbits the intracisternal injection of saline resulted in some degree of pleocytosis. Unmanipulated animals are therefore preferable as controls. Epileptogenic EEG-changes was the most precise of the two variables used for demonstration of neurotoxicity. EEG-changes were therefore used as neurotoxicity criterion in the following rabbit experiments. To evaluate the effect of uraemia alone and uraemia plus meningitis on the neurotoxicity of BPC in rabbits, cephaloridine was used to induce uraemia. Meningitis was induced by intracisternal inoculation of a cephalosporin resistant strain of *E. cloacae*. Untreated rabbits were used as controls. Uraemia resulted in increased BTF penetration of BPC, possibly explained by permeability changes in the BBB and/or decreased binding of BPC to albumin. Uraemia did not result in increased penetration of BPC into the CSF of non-meningitic rabbits. Uraemic non-meningitic rabbits had the highest BTF levels of BPC at the criterion, indicating that cephaloridine-induced renal failure increased the epileptogenic threshold in these rabbits. The combination of uraemia and meningitis increased the neurotoxicity of BPC since the criterion was reached at considerably lower BTF levels of BPC. Meningitis, either alone or together with uraemia, did not increase the neurotoxicity in comparison to control rabbits. Higher BTF levels of BPC were found in meningitic rabbits than in controls with intact blood-CNS barriers at onset of EEG-changes. In all groups of rabbits there was a pronounced variability of BPC levels in the CSF while the intra-group variations in BTF levels were much smaller. Thus, BTF and not CSF levels were decisive for the neurotoxicity of BPC. Using the same EEG-model, the neurotoxic potential of imipenem/cilastatin (I) and a new penem derivative, FCE 22101 were compared in a cross-over study. Both I and FCE 22101 were significantly more neurotoxic than BPC. While BTF levels of the three antibiotics could be detected in all tested rabbits, detectable CSF levels were only found in one of twelve rabbits treated with I or FCE 22101, indicating that BTF concentrations rather than CSF ones are decisive for neurotoxicity of β -lactam antibiotics. The EEG-model used was found to be a suitable model for cross-over studies of intravenously administered antibiotics. Using the "silent-second" as EEG-threshold, a CNS interaction between intraperitoneally administered BPC and intravenous thiopental was demonstrated in rats. The most probably site for this interaction is the organic acid transport system out of the CNS. Thiopental distribution in the rat brain seemed to depend not only on its lipid solubility.

Key words: benzylpenicillin, blood-brain barrier, β -lactam antibiotics, CSF, CNS, EEG, imipenem/cilastatin, FCE 22101, meningitis, neurotoxicity of, silent-second, thiopental, transport system, uraemia.

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To the memory of
Ricardo

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Abbreviations

BBB	Blood-brain barrier
BTF	Brain tissue fluid
CNS	Central nervous system
CSF	Cerebrospinal fluid
EEG	Electroencephalogram
ECF	Extracellular fluid
ECS	Extracellular space
im	Intramuscular
ip	Intraperitoneal
iv	Intravenous

Original papers

This thesis is based on the following papers, which will be referred to by their Roman numerals:

- I Neurotoxicity of benzylpenicillin: correlation to concentrations in serum, cerebrospinal fluid and brain tissue fluid in rabbits.
Schliamser, S. E., Bolander, H., Kourtopoulos, H & Norrby, S.R.
Journal of Antimicrobial Chemotherapy (1988) **21**, 365-372.

- II Neurotoxicity of Benzylpenicillin in Experimental Escherichia coli Meningitis.
Schliamser, S. E., Bolander, H., Kourtopoulos, H., Sigaard, J. & Norrby, S.R.
Journal of Antimicrobial Chemotherapy (**in press**).

- III Neurotoxicity of Benzylpenicillin in Experimental Renal Failure and Enterobacter cloacae Meningitis.
Schliamser, S.E., Bolander, H., Broholm, K. A., Gerdes, U., Kourtopoulos, H. & Norrby, S.R.
(Submitted for publication).

- IV Interaction between Benzylpenicillin and Thiopental in the Central Nervous System of the male rat.
Schliamser, S. E., Karlsson, K., Larsson, J. E., Marklund, S., & Wahlström, G.
Pharmacology & Toxicology (**in press**).

- V Comparative Neurotoxicity of Benzylpenicillin, Imipenem/Cilastatin and FCE 22101, a new injectible Penem.
Schliamser, S. E., Broholm, K. A., Liljedahl, A. L. & Norrby, S. R.
Journal of Antimicrobial Chemotherapy (**in press**).

Introduction

Introduction to the concept of a blood-brain barrier

As early as 1885 Paul Ehrlich had noted that the central nervous system (CNS) differed from other organs in that it was not stained by an intravenously administered aniline dye. Ehrlich's erroneous interpretation was that the brain had a lower affinity for the dye than other tissues. Later, Goldmann (1909, 1913), an associate of Ehrlich, made a detailed study of the vital staining of the tissues of several small mammals injecting the acid dye trypan blue intravenously and into the subarachnoidal space. In the first experiment, after intravenous administration, the dye was widely distributed in most tissues but the brain, spinal cord and peripheral nervous system remained unstained. A lack of uptake of trypan blue was also observed in the foetus after injection to the mother, although the placenta accumulated large amounts of the dye. This finding was suggestive of an analogy between the choroid plexus and the placenta, both exercising a selective and protective function for the tissues beyond them. In the second series of experiments, Goldmann (1913) injected the dye directly into the CSF of rabbits and dogs. In contrast to the lack of symptoms after systemic administration, convulsive movements followed by hyperextension of the limbs and back were observed. Administered by this route, the dye readily stained the brain but did not enter the bloodstream and other internal organs remained unstained.

Thus, Goldmann showed that the CNS is separated from the blood by some kind of a barrier. This barrier, could however, be circumvented by direct injection into the cerebrospinal fluid. From these results, the concept of a haematoencephalic barrier was later postulated. By 1933, Walter and Spatz made a clear distinction between the blood-brain barrier (BBB) and the blood-CSF barrier. However, the term "blood-brain barrier" has often been used in the literature to denote both the barrier between blood and CSF and the barrier between brain tissue and blood. For many years, the barrier was considered to be an anatomical one that totally restricted the passage of certain substances into the brain. With tracer methodology it became clear that most substances were not absolutely excluded from the brain but rather that their rates of entry were reduced in comparison to other organs. The existence of two barriers between blood and CNS which differ from each other both anatomically and functionally is today recognized (Dobbing, 1964, Schanker, 1966).

The BBB consists of the walls of the brain capillaries and the surrounding layers of glia cells while the blood-CSF barrier is formed by the basal membrane of the choroidal epithelium and the tight junctions (zonulae occludens) between the epithelial cells (Schanker, 1966). The capillaries of the CNS do not everywhere have continuous endothelia with tight junctions. Fenestrated endothelia permit transcapillary exchange of protein and other soluble compounds in special regions which do not share the ordinary functions of the major part of the brain. Cells in these regions differ from neurons and require direct contact with blood since they secrete hormones (King, 1938).

As reviewed by Davson (1967), in certain regions of the CNS the blood vessels appear to be permeable to trypan blue, namely the posterior lobe of the hypophysis, the tuber cinereum, the area postrema, the epiphysis or pineal body, the paraphysis, the wall of the optic recess and the eminentia sacularis of the hypophyseal stem.

An absence of barrier or an immature one has also been suggested in the foetus and newborn. Behnsen (1926, 1927) demonstrated that trypan blue injected to young mice was accumulated extensively in the nervous tissue and he deduced from his observations that the BBB was not fully developed in immature animals. The defects in the BBB in young mice were essentially exaggerations of those normally observed in the adult, that is, they were expansions of the above mentioned localized special areas that normally permit the passage of the dye from the bloodstream.

Factors affecting the kinetics of drugs in the CNS

Transport into and out of the CNS of compounds which have physiological or pharmacological activity depends on physico-chemical characteristics identical to those determining the movements of all soluble compounds across biological membranes. However, several factors limit the penetration of drugs into the CNS:

Lipid solubility One of the earliest physical properties recognized to affect the entry of drugs into the CNS was lipid solubility. The degree of lipid solubility of a compound is usually expressed in terms of a partition coefficient which describes the ratio of drug concentration present in an organic phase to that present in a water phase. Good correlation has been found between the lipid solubility of a drug and its rate of entry into the CNS (Brodie, Kurz & Schanker, 1960). Since the capillary bed of the choroid plexus and the meninges is non-fenestrated, all organic compounds have to pass a lipo-protein membrane (Schanker, 1966). Thus, the lipid solubility of a drug influences its ability to cross the blood-CNS barriers (Sande et al., 1978). A high degree of lipid solubility does not however, guarantee good penetration (Norrby, 1978).

Ionization The ionization of a drug is described as the pK , which is equivalent to the pH at which 50 % of the drug is ionized. The degree of ionization of a drug influences the lipid solubility, which is highest for the non-ionized molecules (Barlow, 1964; Bergan, 1978; Norrby, 1978). For example fusidic acid, a highly lipophilic drug, penetrates poorly to the CNS since it is ionized at physiological pH (Gotfredsen, Roholt & Tybring, 1962).

pH gradient Rall, Stabenau & Zubrod (1959) studying a series of drugs administered to normal, acidotic and alkalotic dogs, found that the distribution of weakly ionized and lipid soluble drugs between blood and CSF was also dependent on the pH gradient established between these two compartments. An increased ionization in CSF will lead to improved penetration over the barrier and a decreased ionization to a reduced penetration since the diffusion gradient is maintained between the non-ionized fractions on either side of the barrier. Small changes in the normal pH -gradient of 0.08 between blood and CSF may therefore affect the penetration of a drug and lead to significant changes in the drug distribution ratio.

In presence of meningitis, resulting in lowered pH in the CSF, there is an increased CSF penetration of some antibiotics due to an increased pH-gradient (Norrby, 1979).

Protein binding A wide variety of inorganic and organic compounds are known to interact with plasma proteins (Fingl & Woodbury, 1975). Antimicrobial agents that combine with serum proteins do so mainly by forming a loose and rapidly reversible bond with serum albumin. Binding to other serum proteins occurs but to a lesser extent; for example, erythromycin binds to alpha-globulins and tetracycline to lipoproteins (Craig & Suh, 1978a). Since only free, unbound drug can pass through the capillary pores into tissue fluids, drugs which are highly bound to serum proteins tend to remain in the intravascular compartment giving high blood levels but low tissue levels. However, if the drug is lipid soluble, it can pass through the cell membranes. Kunin (1965) showed that the brain levels of some penicillins were inversely related to the amount of drug bound to plasma protein and that orthocresotinic acid, which prevents binding of penicillin to rabbit albumin, also increased the penetration of ampicillin into the CSF. Increased passage of benzylpenicillin through the blood-CSF barrier has been shown by Spector & Snodgrass (1976) in experimentally induced uraemia, resulting in a lower degree of protein binding of benzylpenicillin. The isoxazolympenicillins are highly protein bound and penetrate poorly the CNS (de Louvois, Gortvai & Hurtle, 1977).

Molecular size and structure Compounds having high molecular weight and complex structure, such as polymyxin B, penetrate poorly into the CNS even in the presence of inflammation (Lerner, 1974). Aminoglycosides which also have a complex structure have a low capacity to cross the blood-CSF barrier (Goiten, Michel & Sachs, 1975).

Meningeal and brain tissue inflammation As pointed out by Oldendorf (1975), the permeability of the blood-CNS barriers is increased in virtually all lesions of the CNS in which normal histology is significantly altered. Inflammation increases capillary permeability and disrupts tight junctions, for example in the choroid plexus. There may also be alterations of local transport regulating mechanisms. Consequences of the breakdown of the blood-CSF barrier are increased CSF protein concentration and an accelerated penetration of substances which normally enter the CSF very slowly after

injection into the bloodstream, for example, iodide, nitrate and fluorescein. Davson & Smith (1957) studied the effects of inflammation on the permeability of the blood-CSF barrier using the bromide test for calculation of a "permeability quotient" between plasma and CSF and found consistent deviations from the normal state in inflammatory conditions of the CNS.

Several studies have shown increased penetration of antibiotics through the blood-CSF barrier in the presence of meningeal inflammation (Lithander & Lithander, 1962; Sande et al., 1978). In experimental meningitis, Ruedy (1965) demonstrated significantly increased levels of penicillins in the CSF of rabbits and a high correlation between penicillin CSF concentration and the severity of meningitis as judged by the CSF protein content. Lithander (1964) demonstrated a marked variation in the CSF antibiotic penetration depending on the infecting bacterial species. The highest CSF levels were obtained with streptococcal infections and the lowest in staphylococcal infections. In human studies, several-fold increases of the concentrations of many antibiotics such as cefuroxime (Muller & Netland, 1980; Norrby et al., 1982), ampicillin (Geering & Just, 1975), thrimetoprim/sulphamethoxazole (Sabel & Brandberg, 1975) have been demonstrated in the CSF of patients with meningitis. In human meningitis, Barrett et al. (1966) showed a correlation between CSF antibiotic levels and the degree of CSF pleocytosis as well as CSF protein concentration.

Very few studies have been performed on the significance of inflammation for the penetration of antimicrobial agents into intracranial abscesses. In experimentally induced abscesses it was shown that steroids reduce the penetration of lipid-insoluble antibiotics such as benzylpenicillin while no negative effects were noted for the penetration of lipophilic ones such as metronidazol (Kourtopoulos, Holm & Norrby, 1983a). In a human study performed on neurosurgical patients, the penetration of chloramphenicol into brain tissue was not affected by concomitant steroid treatment (Kramer, Griffith & Campbell, 1969). Holm & Kourtopoulos (1985) suggested that lipid insoluble antibiotics have to be given in higher doses when steroids are given simultaneously since steroids may effectively hinder penetration into infected tissues.

Active transport mechanisms The existence of an efflux transport mechanism within the CSF was first suggested by Pappenheimer, Heisey & Jordan (1961) based on the finding that iodopyracet (Diodrast) and phenosulfonphthalein (PSP) were transported from CSF to blood against a concentration gradient.

Sampath & Neff (1973) demonstrated the existence of a transport system in the choroid plexus for elimination of 5-hydroxyindolacetic acid (5-HIAA), the acidic product of serotonin metabolism, from the CSF to blood. Probenecid, cephaloridine and indolacetic acid were able to inhibit the uptake of 5-HIAA. Neff, Tozer & Brodie (1967) could demonstrate inhibition of the efflux of 5-HIAA from rat brain by probenecid. Several studies have shown that benzylpenicillin is actively transported out of the CSF and that transportation can take place against a concentration gradient (Fishman, 1966; Dixon, Owens & Rall, 1969; Pollay, 1974). Probenecid has been shown to inhibit the transportation both in experimental animals and in man (Dewhurst, 1969; Dacey & Sande, 1974; Spector & Lorenzo, 1974; Walters, et al., 1976; Kourtopoulos, Holm & Norrby, 1983b). Active transport mechanisms have been implicated in facilitating the entry into brain of a number of metabolites, such as glucose (Crone, 1965) and amino acids (Lajtha, 1962). Furthermore, it has been suggested that active transport mechanisms may facilitate the transfer of substances from brain to blood (Lajtha, 1962; Oldendorf, 1975).

Cerebral circulation Neither regional blood-flow nor vascularity is uniform within brain (Barlow, Schoolar & Roth, 1958; Freygang & Sokoloff, 1958). Differences in the blood-flow rate in different regions of the brain may contribute to different drug levels at various sites in the brain.

CSF production and circulation The total CSF volume in an adult man is approximately 140 ml and the ventricular CSF volume 23 ml. The mean CSF production has been calculated to be 0.3-0.4 ml/min or 500-600 ml/day (Wood, 1980). Approximately 0.25% of the total CSF volume is replaced by freshly secreted fluid every minute; thus total CSF volume is renewed every 5-7 hr. The choroid plexus accounts for approximately 70% of CSF production, whereas the remaining CSF is derived from the capillary bed of the brain and metabolic water production. CSF production rate, flow rate and flow direction are factors affecting the concentrations of drugs in the CSF. DiChiro (1964) could detect radiolabelled albumin in the basal cisterns approximately one hour after injection into the lumbar sac of man, and 20% to 33% of the isotope reached the intracranial cavity within 12 hours. When injecting the radioisotope-labelled albumin intraventricularly in man, the radioactivity reached the basal cisterns within a few minutes and began to pass through the anterior subarachnoid spaces and Sylvian fissures at two hours to concentrate along the superior sagittal sinus area at 12 to 24 hr.

In addition, Riesselbach et al. (1962) demonstrated that following lumbar administration, a compound could be detected in the basal cisterns if the volume injected was 10% of the total CSF volume. Measurable concentrations reached the ventricles when the volume injected into the lumbar space was 25% of the total CSF volume.

Following intralumbar administration, high levels of gentamicin could be assayed in lumbar CSF while the concentrations assayed in ventricular CSF were extremely low. If gentamicin was given intraventricularly high levels could be demonstrated in both ventricular and lumbar CSF (Moellering & Fisher, 1972). Concentrations of benzylpenicillin following systemic administration have been shown to be higher in ventricular and cisternal CSF than lumbar CSF (Dumoff-Stanley, Dowling & Sweet, 1946).

The extracellular space of the brain (ECS) The size of the brain ECS has been extensively discussed. Using light microscopy, the brain appeared to contain a large amount of extracellular fluid (ECF). It was assumed that the sodium and chloride ion concentrations in brain were valid measures of the brain ECF space (about 30 % to 35 %). However, both light microscopy and calculations of brain tissue electrolytes, such as sodium and chloride, have been found to be misleading for estimation of brain ECS. Electron micrographs of brain have shown that the non-neuronal areas of brain are filled with glial cells and that what appears to be ECF in light microscopy is the "watery" cytoplasm of the glia, particularly of the astrocytes. The brain ECF space has been estimated to be 3% to 4% by electron microscopists (Maynard, Schultz & Pease, 1957). This is in agreement with the inulin and sulphate spaces after parenteral administration of these compounds (Barlow et al, 1961). However, estimates based on electron micrographs have been considered to underestimate brain ECF, since glial swelling, resulting from tissue anoxia before fixation, encroaches on the ECF as shown by Van Harreveld, Crowell & Malhotra (1965). Using physiological indicators injected systemically, spaces as large as 10% to 15% of the net tissue weight have been measured (Rall, Oppelt & Patlak, 1962; Cutler, Deuel & Barlow, 1967). In spite of considerable controversy, it now seems clear the existence of an ECF space of brain larger than the space estimated by electron microscopists (Fenstermacher & Patlak, 1975; Oldendorf, 1975).

The size of the brain ECS is an important factor for the steady-state concentration of drugs restricted to an extracellular compartment, that is, water soluble and non-lipophilic substances which are not actively transported to cells.

Neurotoxicity of beta-lactam antibiotics

The neurotoxic effect of penicillin has been known since 1945 when Johnson & Walker reported the first case of myoclonic twitches and coma following intraventricular administration to a boy with "Staphylococcus albus" ventriculitis. Experimental studies confirmed the neurotoxicity of penicillin after intracisternal administration or application to the cerebral cortex of cats, monkeys and man (Walker & Johnson, 1945; Walker, Johnson & Kollros, 1945; Borkowski & Forster, 1948). Neyman, Heilbrunn & Youmans (1945) described generalized muscular twitchings and finally continuous convulsions and death after penicillin treatment of patients with neurosyphilis by the intracisternal route. Philippe & Goutorbe (1948) reported a fatal case of seizures following intrathecal administration to a baby. The first reports of penicillin neurotoxicity following intravenous administration appeared in the 1960ies (Weinstein, Lerner & Chew, 1964; New & Wells, 1965; Oldstone & Nelson, 1966; Lerner, Smith & Weinstein, 1967). Since then, reports of severe neurotoxic side effects of penicillin have continued to appear, mostly in patients with impaired renal function (Bloomer, Barton & Maddock, 1967; Cohill et al., 1967; Grenvik & Wigren, 1968; Lavy & Stein, 1970; Love & Salter, 1972; Fossiek & Parker, 1974) and patients undergoing open heart surgery with cardiopulmonary bypass (Dobell et al., 1966; Borman & Eyal, 1968; Seamans et al, 1968; Gloor, 1969; Currie et al., 1971). Experimental studies in dogs confirmed the neurotoxicity of penicillin during thoracic surgery with cardiopulmonary bypass, but in those studies neither the bypass procedure alone nor large doses of penicillin alone were able to produce seizures (Wyant & Dobell, 1967; Currie et al., 1970). Seamans et al. (1968) and Gloor (1969) suggested a breakdown of the "blood-brain barrier" during cardiopulmonary bypass and a subsequently increased penetration of penicillin over the barrier as the possible mechanisms responsible for neurotoxicity.

In contrast to benzylpenicillin, ampicillin was not found to cause neurotoxic symptoms when infused in large doses to rabbits (Weihrauch, 1974). Raichle et al. (1968) studied the effects of methicillin, oxacillin and ampicillin on the CNS of cats. None of them induced neurotoxic reactions after intravenous administration. When the drugs were applied intracortically, all were able to produce epileptogenic effects but considerably less potent than

benzylpenicillin. Gerald, Massey & Spadaro (1973) in a comparative study of the convulsant activity of various penicillin derivatives and cephaloridine given by intracerebral injection to mice, found that penicillin G was most neurotoxic. The rank order of the neurotoxicity of the other drugs in descending order was: methicillin > cephaloridine > phenoxymethylpenicillin and ampicillin, the two latter being one-fifth as toxic. Weihrauch, et al. (1975) showed that penicillin induced characteristic EEG-changes: spikes, spike and wave paroxysms and polyspikes. Pheneticillin, oxacillin, cloxacillin and dicloxacillin precipitated general discharges followed by generalized convulsions as described for benzylpenicillin. When comparing the effects on the EEG of various penicillins, benzylpenicillin showed the highest neurotoxic potency.

Carbenecillin has also been reported to precipitate neurotoxicity following intravenous administration to patients (Kurtzman, Rogers & Harter, 1970; Blum & Matsen, 1971).

Cephalosporins have rarely been associated with seizures, but there are reports of neurotoxic manifestations after both intravenous and intrathecal administration. In 1977, the first report of cefazolin-induced seizures appeared in the literature (Yost, Lee & O'Leavy, 1977). Moore, Bechtel & Ayers (1981) studied the relationship between serum and CSF concentrations of cefazolin and their association to neurotoxic reactions in patients with intact blood-CSF barriers receiving multidose therapy with cefazoline. They could confirm a neurotoxic potential of the drug associated with renal dysfunction and multiple dose therapy. Also cephaloridine has been reported to produce seizures after intrathecal administration to patients (Yoshioka et al., 1975). However, high dose cefuroxime has mistakenly been administered intraventricularly to a patient without any adverse effect (Norrby, 1987). Renal failure has been associated with the neurotoxicity of cephalosporins (Wu et al., 1978; Bechtel, Slaughter & Moore, 1980). Severe myoclonus has been reported under moxalactam treatment (Bertoni & Cho, 1983).

Neurotoxic reactions to newer beta-lactam antibiotics have been reported in clinical trials of imipenem in combination with cilastatin, an inhibitor of the renal metabolism of imipenem. They have mostly been seen in patients with decreased renal function and/or those receiving large doses of imipenem/cilastatin relative to age, renal function and body weight. The

incidence of such manifestations varies from 0.4 % to 7.5% in different studies (Calandra et al., 1985; Solomkin et al., 1985; Park & Parker, 1986; Tse, Hernandez Vera & Desai, 1987). Brotherton & Kelber (1984) reported two cases of seizure-like activity associated with imipenem/cilastatin. They suggested decreased renal function, accumulation of imipenem after multiple dosing and/or a probenecid-like effect of cilastatin on the CSF excretion of imipenem, as possible mechanisms responsible for the neurotoxicity of the drug. The latter proposal was based on the fact that cilastatin has a probenecid-like effect on the renal excretion of imipenem.

Pathogenesis of neurotoxicity

The pathogenesis of neurotoxic reactions to beta-lactam antibiotics is not clarified. The action of penicillin has been studied extensively by neurophysiological techniques and it's generally held that the convulsant action results from alterations in synaptic transmission (Cutler & Young, 1979). For penicillins, a possible mechanism may be excitation of the CNS neurons by a blockade of the synaptic activity of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), possibly at the GABA-receptor level (Macdonald & Barker, 1978). Antoniadis, Muller & Wollert (1980) demonstrated affinity of various penicillins for the benzodiazepine receptor inhibiting specific [3 H]flunitrazepam binding to that receptor in a concentration-dependent fashion. A very good correlation was found between the affinity of the penicillin derivatives for the benzodiazepine receptor as given by the IC₅₀ values (concentrations needed to inhibit specific [3 H]flunitrazepam binding by 50%) and their neurotoxicity. The authors suggested that besides antagonism at the GABA receptor, antagonistic properties at the benzodiazepine receptor could be involved in the neurotoxicity of penicillin.

Hori et al. (1988) studied the effect of β -lactamase inhibitors on γ -aminobutyric acid receptor bindings. Cefazolin and cefoperazone inhibited GABA-receptor binding in a concentration dependent manner. Ampicillin in high concentrations inhibited GABA-receptor binding.

Gutnik & Prince (1971) studied the effects of penicillinase on the convulsant action of penicillin. Parenteral penicillinase injection significantly shortened the time course of the generalized electrical and behavioural

seizure activity which occurred after administration of large doses of impenicillin to chronic implanted cats. Penicillinase had no effect on spontaneous or flash evoked EEG activities when topically applied to the cortex in concentrations of 400,000 units/cm³. Incubation in vitro of penicillin with penicillinase completely neutralized the capacity of penicillin to produce epileptogenic foci when directly applied to the cortex. Application of penicillinase to already established penicillin epileptogenic foci shortened the time course of the focal discharge. They suggested that the epileptogenic characteristics of penicillin, like its antimicrobial activity are dependent on the β -lactam ring which is hydrolyzed in the presence of penicillinase. In addition, Esplin et al. (1985) suggested that the structural requirements for the epileptogenic activity of benzylpenicillin depends not only on the β -lactam ring and the side chain on C-6 but also, on the thiazolidine ring.

Aims of the Study

The aims of these studies were

1. to develop a rabbit model suitable for studies of the neurotoxicity of intravenously administered antibiotics,
2. to study the neurotoxic potential of benzylpenicillin in normal rabbits,
3. to study the effects of meningeal inflammation and/or uraemia on the neurotoxicity of benzylpenicillin,
4. to study a possible interaction between benzylpenicillin thiopental in the CNS of rats and
5. to compare the neurotoxicity of benzylpenicillin, imipenem/cilastatin and a new injectible penem antibiotic, FCE 22101.

Material and Methods

Animals

All experiments were approved by the Animal Ethics Committee, Umeå. The experiments were performed on healthy adult male or female rabbits (I-III, V) and male Sprague-Dawley rats (IV).

Bacterial strains

The *Escherichia coli* strain used (II) was a human urinary isolate obtained from the Serum Institute, Copenhagen, Denmark. The *Enterobacter cloacae* strain (III) was a human septicaemia isolate resistant to 1st, 2nd and 3rd generation cephalosporines. It was kindly supplied by the Department of Microbiology, University of Umeå.

Antibiotic assays

Different techniques have been used for determination of antibiotic levels in tissues. In body fluids such as sputum, pleural fluid, sinus fluid, etc., microbiological methods are the most common used. In whole tissue assays, homogenization or elution followed by microbiological assay are normally used. Application of whole tissue biopsies on agar plates has been used for bone tissue samples by Dornbusch (1978) who also included electrophoresis to maximize elution. Cars (1981) determined antibiotic concentrations in small muscle tissue specimens by using an agar well diffusion technique. Wellman et al. (1954) were the first to determine levels of antimicrobial drugs in fresh human brain tissue. Kramer et al. (1969) determined the concentrations of several antibiotics in brain tissue of patients undergoing neurosurgery; brain samples were frozen, ground to a powder and suspended in a buffer solution to a standard volume. Kourtopoulos, Holm & Norrby (1983a) determined antibiotic concentrations in brain by using an agar well diffusion technique and applying brain slices on top of preseeded agar plates.

All samples were frozen at -70°C until assayed.

Concentrations of benzylpenicillin in serum, CSF and brain tissue fluid were determined by an agar disc diffusion technique with *Bacillus subtilis* ATCC 12432 as test organism allowing the detection of 0.125 mg/l of benzylpenicillin. All samples were analysed in triplicate and compared with fresh standards diluted in phosphate-buffered-saline (PBS). Ten μl of the standard serum or CSF samples were applied on paper discs having a diameter

of 5 mm. Two standards were included on each agar plate. The frozen brain samples were thawed and sliced, at least six pieces of each sample, each slice weighing between 30 and 40 mg and having a diameter of approximately 5 mm (Figure 1). The brain slices were placed together with the standard discs on agar plates and following incubation overnight, the zones of inhibition were measured and, compared to a standard curve. Serum or CSF samples giving inhibition zones larger than that of the highest standard concentration were retested after dilution in PBS and undiluted together with a new higher standard.

Figure 1. Agar plate showing inhibition zones around paper discs and brain slices.

Control experiments demonstrated that no inhibition zones were obtained with brain discs from untreated rabbits. When standard solutions of benzylpenicillin were added to brain discs from untreated animals, the inhibition zone diameters did not differ from those obtained with paper discs.

To compare the technique used with other methods for biological assays of brain concentrations of benzylpenicillin, a series of experiments were performed. Normal rabbit brain and brain samples from four rabbits treated with benzylpenicillin were homogenized in an Ultra-Turrax homogenizer at room temperature for a few minutes. Aliquots of the homogenate from the untreated rabbit were mixed with benzylpenicillin to give final concentrations of 8 to 512 mg/l. Each homogenate was then applied on paper discs using 0.01 ml volumes per disc and the conditions described above for brain slices. This method gave standard curves described by the formula $y = -2.6 + 0.13x$, R (correlation coefficient) = 1.00 as compared to $y = -2.28 + 0.16x$, $R = 0.97$ for benzylpenicillin applied directly to the discs. The concentrations

assayed with these techniques in brain samples from treated rabbits are given in Table I. Lower concentrations were assayed with the homogenization technique than with the technique used by us. The differences might have been due to the fact that in the homogenate the free protein concentration was considerably higher than in the slices. Moreover there is a likelihood that the homogenization procedure may have inactivated benzylpenicillin. It seems permissible to state that the technique used by oss was the most suitable one for demonstration of the tissue fluid concentrations of benzylpenicillin in brain samples.

Table I. Comparison of benzylpenicillin concentrations assayed in brain tissue using homogenization and application to paper discs or brain slices applied to the agar surface.

Method	Rabbit No. and benzylpenicillin concentration assayed (mg/l or mg/kg)			
	1	7	10	12
Homogenization	4.8	9.0	7.0	6.0
Brain slices	6.5	32.5	12.0	7.6

Several techniques have been used for studies of antibiotic penetration to cells. As shown by Forsgren & Bellahsene (1985) in a study of the penetration of several radiolabelled antibiotics to human polymorphonuclear leucocytes and lymphocytes, the beta-lactam antibiotics acidocillin, ampicillin, benzylpenicillin, cloxacillin and cefoxitime did not accumulate in human leucocytes or lymphocytes, while doxycycline, erythromycine and rifampicin did. These results were in accordance with previous findings for penicillin and rifampicin (Mandell, 1973). However, Brown & Percival (1978) used a direct microbiological assay of cell lysates to detect cell penetration of antibiotics and showed cell-associated levels of benzylpenicillin in HeLa cells. According to Forsgren & Bellahsene (1985), the discrepancies between the results obtained by Brown & Percival (1978) and those of others, can be explained by the use of different techniques; biological methods detect biologically available antibiotic, while techniques using radioactive antibiotics measure total antibiotics. Against that background we preferred

to use brain tissue fluid (BTF) concentrations rather than concentrations in whole brain tissue since benzylpenicillin is a water soluble, lipid insoluble antibiotic.

BTF concentrations were calculated as the amount of benzylpenicillin detected, divided by the weight of the sample and multiplied with a factor 10, using the assumption that the brain tissue consists of about 90% cellular elements and 10% extracellular fluid (Rall, Oppelt & Patlak, 1962; Rall & Zubrod, 1962; Rall, 1971).

Imipemem/cilastatin and FCE 22101 concentrations were determined with the same method as above with imipenem and FCE standards.

Cephaloridine concentrations were assayed using *B. subtilis* and as an extra control a penicillinase producing strain of *Staphylococcus aureus* as test organisms.

Determination of thiopental concentrations in serum and brain tissue

The method for analysis of thiopental used in IV is a one- step extraction procedure combined with high pressure liquid chromatography (HPLC) determination, essentially described by Bolander, Wahlström & Norberg (1984).

Brain samples were homogenized, weighed and acidified by addition of 2M NaH_2PO_4 . Serum samples were mixed with an equal volume of 2M NaH_2PO_4 . All samples were eluted with ethylacetate containing another barbiturate as internal standard. After shaking for 30 sec the samples were allowed to stand for 20 min and were then centrifuged. The supernatant was dried under a stream of nitrogen. The dry residue was dissolved in methanol, and 20 μl were injected into a HPLC (LDC model 3 Constametric) fitted with a reversed phase column and a variable UV-detector. The mobile phase was a mixture of methanol and water (60:40) and the flow rate was 1.3-2.0 ml/min. The UV-detector was set at either 280 nm, and a standard curve was made simultaneously by analysing known amount of barbiturate added to serum or brain tissue homogenates.

Determination of haemoglobin content in brain tissue

Blood contamination was determined in brain tissue of rats (IV) using a previously developed method originally devised for human haemoglobin based on the specific binding of haemoglobin by haptoglobin and the stabilizing effect of this binding on the peroxidase activity of haemoglobin (Marklund, 1979).

The haemoglobin containing solution is incubated under denatured conditions in the presence of haptoglobin (serum) or a protein solution without haptoglobin (egg white). The difference in peroxidase activity between samples incubated with serum or with egg white is proportional to the amount of haemoglobin. This method was found to be applicable to rat haemoglobin as well.

Neurophysiological methods

The electroencephalographic (EEG) method.

Antibiotics (except cephaloridine) were infused in rabbits through an ear vein by an infusion pump (Ed II Braun, Zurich, Switzerland). EEG was recorded continuously from implanted screw (I) or subcutaneous stainless steel wire suture electrodes (II, III, V) with an ordinary EEG-recorder (Mingograf EEG, Siemens-Elema, Södertälje, Stockholm, Sweden) until the appearance of epileptogenic activity defined as three or more spikes and/or spikes and waves during a period of ≤ 20 sec or clinical convulsions (I, II). The infusion was immediately stopped and the time and amount of drug needed to reach the criterion were recorded and samples of blood, CSF and brain tissue were collected for determination of antibiotic levels.

The EEG-threshold method ("silent-second").

In (IV) the experiments were performed on rats pretreated with benzylpenicillin intraperitoneally and then infused with thiopental through a tail vein with a constant rate during continuous EEG-recording until the appearance of the first burst suppression period of 1 sec or longer (the "silent-second") in the EEG. When the first "silent-second" was recorded, the infusion was stopped, the rats were immediately sacrificed and the time and amount of thiopental used to reach the criterion of silent-second were registered. Blood and brain samples were collected for determination of thiopental and benzylpenicillin. This EEG-threshold method has been developed by Wahlström (1966) and is mainly used for estimation of potency or sensitivity to anaesthetic barbiturates using an optimal dose rate (Bolander, Wahlström & Norberg, 1984).

Experimental design

The first study (I) was performed on rabbits with intact blood-CNS barriers. Benzylpenicillin was administered through an ear vein using an infusion pump at a dose rate of 10 mg/kg/min during the first hour, 20 mg/kg/min during the second hour and 40 mg/kg/min during the third hour. EEG was

recorded during infusion until the appearance of epileptogenic activity in the EEG or convulsions.

The second study (II) was performed on rabbits with experimentally established *E. coli* meningitis after inoculation of bacteria into the cisterna magna. This procedure was done during anaesthesia. At least 24 hr elapsed between intracisternal inoculation and EEG-recording. Benzylpenicillin was administered as in the first experiment (I).

The third study (III) was performed on rabbits with experimental renal failure secondary to cephaloridine-induced acute tubular necrosis and rabbits with intact meninges or experimentally established *E. cloacae* meningitis. Cephaloridine dissolved in saline was administered through an ear vein 48 hr before benzylpenicillin treatment. Benzylpenicillin was infused during EEG-recording at a constant dose rate of 10 mg/kg/min until the onset of epileptogenic activity in the EEG as previously defined.

In (IV) the experiments were performed on rats pretreated with benzylpenicillin administered intraperitoneally and then infused through the tail vein with thiopental until the appearance of the first "silent-second" (criterion).

The fifth series of experiments (V) was a cross-over neurotoxicity study in rabbits using benzylpenicillin, imipenem/cilastatin and FCE 22101 as test-drugs. All antibiotics were administered during EEG-recording. At least 7 days elapsed between antibiotic administrations. Benzylpenicillin was administered at a rate of 10 mg/kg/min, for the first hour, 20 mg/kg/min for the second. Imipenem/cilastatin and FCE 22101 were given at a rate of 1.25 mg/kg/min during the first and 2.5 mg/kg/min during the second hour of administration.

Results and Discussion

Benzylpenicillin Neurotoxicity in Rabbits with Intact Blood-CNS Barriers (I)

This study aimed at evaluating the neurotoxic potential of intravenously administered benzylpenicillin in normal rabbits.

The main findings are summarized in Table II.

Table II. Comparison between doses and kinetic parameters at onset of epileptogenic EEG-changes and seizures in rabbits with intact blood-CNS barriers. All values are given as means $\pm$ SEM.

Parameter	Criterion	
	EEG-changes	Seizures
	(N=7)	(N=6)
Benzylpenicillin dose (mg/kg)	550 $\pm$ 102	1767 $\pm$ 566
Time to the criterion (min)	51 $\pm$ 7	103 $\pm$ 21
Serum concentration (mg/l)	707 $\pm$ 63	1282 $\pm$ 134
CSF concentration (mg/l)	1.28 $\pm$ 0.48	6.0 $\pm$ 1.6
Relative CSF penetration* (%)	0.18 $\pm$ 0.05	0.5 $\pm$ 0.15
BTF concentration (mg/l)	108 $\pm$ 16	243 $\pm$ 59
Relative BTF penetration* (%)	15.5 $\pm$ 1.8	19.6 $\pm$ 4.9

*% of concurrent serum concentrations

The onset of convulsions was seen at various times after the onset of epileptogenic EEG-activity. The doses of benzylpenicillin needed to achieve the criterion in this group varied between 500 and 3680 mg/kg, while the serum concentrations varied less markedly and were between 920 mg/l and 1902 mg/l. The CSF and brain tissue fluid concentrations assayed in this group were significantly higher ($p < 0.01$ and $p < 0.05$ respectively) and more variable than those found in rabbits sacrificed at onset of epileptogenic EEG-activity. There was no significant difference between the groups with regard to the relative penetration to CSF and brain tissue fluid. However, in

all rabbits, the BTF concentrations of benzylpenicillin were much higher than those assayed in CSF.

In order to elucidate the kinetic mechanism(s) responsible for the neurotoxicity of benzylpenicillin, correlations between the studied variables were calculated. In rabbits sacrificed at onset of epileptogenic EEG-activity, poor correlations were found between the total doses administered and the concentrations of benzylpenicillin in serum, CSF and brain tissue fluid. However, as shown in Figure 2, a high correlation could be demonstrated between CSF and BTF concentrations of benzylpenicillin at onset of epileptogenic EEG-activity ($R=0.93$, $p < 0.005$). In rabbits sacrificed at onset of seizures the correlation between CSF and BTF concentrations was not significant ($R=0.61$). When correlating CSF and BTF concentrations of benzylpenicillin in all animals at the criteria (epileptogenic EEG and seizures), a high correlation was found ($R=0.78$, $p < 0.005$).

Figure 2. Correlation between benzylpenicillin concentration in brain tissue fluid and CSF in rabbits sacrificed at onset of epileptogenic EEG-activity ($R=0.93$, $p < 0.005$).

A significant correlation was also found between the dose of benzylpenicillin administered and BTF concentrations at the criterion in all rabbits ($R=0.68$, $p < 0.02$). A high correlation ($R=0.87$, $p < 0.01$) was seen between serum and BTF concentrations of benzylpenicillin in rabbits which developed EEG-changes or convulsions with the lower dose-regimen (10 mg/kg/min). No such correlation was seen in animals that required increased doses for precipitation of neurotoxicity.

This study confirmed previous findings that intravenously administered benzylpenicillin has a neurotoxic potential in rabbits with intact blood-CNS barriers (Weihsrauch, et al., 1974). Our findings were in accordance with those of Weihsrauch's who stated that blood levels of benzylpenicillin of 1000 units/ml (600 mg/ml) or higher caused neurotoxicity in the rabbit. In that

study no concurrent CSF or brain tissue concentrations of benzylpenicillin were assayed.

The BTF levels of benzylpenicillin were very much higher than the CSF concentrations. This is in agreement with findings by Lithander & Lithander (1962) who demonstrated higher concentrations of benzylpenicillin in the brain than in the CSF of control rabbits. These findings may be the result of an active transport of benzylpenicillin out of the CSF space. Such a transport was described for organic acids, such as, Diodrast and phenolsulfonphtalein (PSP) by the pioneer work of Pappenheimer, Heisey & Jordan (1961) using ventriculocisternal perfusion. They also suggested the possibility of a similar transport for removal of penicillin from CSF to blood, since penicillin is an organic acid which, similar to Diodrast, PSP and p-aminohippurate (PAH), is secreted by the proximal tubules of the kidney. The existence of such a transport system for benzylpenicillin was described later by Fishman (1964, 1966) and Dixon, Owens & Rall (1969) who found it to be an active, saturable transport against a concentration gradient and which could be competitively inhibited by other organic acids like probenecid and PAH. The saturability of the transport system may contradict the hypothesis that this is the only explanation for our findings of higher BTF than CSF concentrations of benzylpenicillin. Thus, no significant differences were found for the relative penetration to CSF and to BTF between rabbits sacrificed at onset of epileptogenic EEG-activity and those sacrificed later at onset of convulsions.

Another complicating factor in evaluation of the kinetics of benzylpenicillin in the CSF is the suggestion that there exist a saturable active transport for entry into the CSF. Lithander & Lithander (1962) could not show increases of the CSF concentrations of benzylpenicillin which were proportional to the increases of plasma concentrations in rabbits.

A better penetration of benzylpenicillin over the BBB or a higher affinity for brain tissue is another possible explanation for the higher BTF than CSF concentrations of benzylpenicillin. Lorenzo & Spector (1976) suggested the existence of non-specific binding sites for penicillin in brain.

No correlations were found between CSF concentrations of benzylpenicillin and onset of neurotoxicity. This indicates that, in rabbits with intact meninges, the concentrations of benzylpenicillin in the brain, rather than in CSF, are decisive for precipitation of neurotoxicity. Our results are in agreement with those presented by Quesney & Gloor (1978) who studied the distribution of ^{14}C -penicillin in experimental feline generalized epilepsy after administration of large doses using different routes during continuous

EEG-recording from cortical and subcortical structures. They found significantly higher levels of penicillin in cortex, rather than in CSF of cats at onset of generalized epileptic activity after intramuscular administration. The authors concluded, as previously suggested by Gloor, Quesney & Zumstein (1977) and Fisher & Prince (1977), that the alteration in neuronal function responsible for the epileptiform features of feline generalized penicillin epilepsy is located to the cerebral cortex and is caused primarily by a cortical effect of penicillin and not by effects on subcortical structures.

Our observations that high BTF levels of benzylpenicillin seemed correlated to high serum levels is in agreement with the suggestion by Lithander & Lithander (1962) that, in rabbits with intact blood-CNS barriers, benzylpenicillin enters the brain mainly by direct passage from the blood. This supports the hypothesis that the CNS penetration of benzylpenicillin takes place over two different barriers; the blood-CSF and the blood-brain barrier.

More pronounced inter-animal variations of serum and BTF levels were found in rabbits studied after onset of convulsions and in rabbits which required increased doses of benzylpenicillin for induction of neurotoxicity. These observations may be explained by individual variations in the threshold for convulsions. Thus, epileptogenic EEG-changes seem to be the more precise variable for demonstration of neurotoxicity.

The method developed, constant infusion of benzylpenicillin to a distinct EEG-criterion, was shown to be a suitable method for experimental neurotoxicity studies.

Neurotoxicity of Benzylpenicillin in Experimental E. coli Meningitis (II)

The neurotoxic potential of intravenously administered benzylpenicillin was studied in rabbits with experimentally induced E. coli meningitis. As controls, a group of rabbits was injected with saline into the cisterna magna. In all rabbits inoculated with E. coli, meningitis was verified by CSF pleocytosis ($>1000 \times 10^6$ leukocytes/l), positive bacterial cultures from the CSF and by microscopical examination. In the control group, a varying degree of moderate pleocytosis was found ($<100 \times 10^6$ leukocytes/l), but none of the control rabbits presented clinical or histological signs of inflammation in the CNS and none of them yielded positive bacterial CSF cultures.

Tables III and IV give the main findings of the study.

Table III. Comparison between doses and kinetic parameters at onset of epileptogenic EEG-changes and seizures in rabbits with experimental *E. coli* meningitis and controls. All values are given as means $\pm$ SEM.

Group and criterion at time of sacrifice	Time(min) to the criterion	Kinetic parameter			
		Total dose (mg/kg)	Benzylpenicillin Serum CSF	concentration in the BTF (mg/l)	
Meningitis					
EEG-changes	66	799	2491*	119	214#
	±14	±220	±334	±49	±25
Seizures	55•	748~	2077	202	304
	±21	±302	±376	±64	±72
Controls					
EEG-changes	79	992	1043*	171	134#
	±10	±204	±292	±98	±28
Seizures	148•	2960~	2623	75	560
	±20	±622	±841	±52	±127

Symbols denote statistical significances *, ~ $p < 0.01$ and •, # $p < 0.02$

Table IV. Relative penetration to CSF and BTF in rabbits with *E. coli* meningitis and controls sacrificed at onset of epileptogenic EEG-changes or seizures. All values are given as means $\pm$ SEM.

Group and criterion at time of sacrifice	Relative penetration	
	CSF/Serum (%)	BTF/Serum (%)
Meningitis		
EEG-changes	6.36 $\pm$ 3.26 (6)	9.05 $\pm$ 1.23 (6)
Seizures	8.67 $\pm$ 2.54 (5)	14.28 $\pm$ 2.29 (6)
Controls		
EEG-changes	14.87 $\pm$ 8.26 (6)	19.84 $\pm$ 5.88 (7)
Seizures	3.95 $\pm$ 2.74 (4)	25.18 $\pm$ 4.71 (4)

Figures within brackets indicate number of rabbits

When comparing the meningitis and the control groups, at onset of epileptogenic EEG-changes, the latter had significantly higher ($p < 0.05$) relative penetration to the BTF. This group also had significantly higher ($p < 0.05$) relative penetration to BTF than to CSF. The time and the doses of benzylpenicillin needed to induce epileptogenic EEG-changes and seizures were significantly lower ($p < 0.05$ and $p < 0.02$, respectively) in meningitic than in uninfected rabbits. No obvious differences were found between serum, CSF and BTF concentrations of benzylpenicillin, but the relative penetration to BTF was higher in the controls. Rabbits sacrificed at onset of seizures had significantly higher ($p < 0.01$) concentrations of benzylpenicillin (both absolute and relative) in BTF than those sacrificed at onset of epileptogenic EEG-changes.

Contrary to data presented by Lithander (1966), we found no significant differences between meningitic and uninfected animals with regard to the CSF penetration of benzylpenicillin. However, Lithander administered

considerably lower doses as a bolus iv injection and all rabbits were sacrificed 30 min after injection. Another possible explanation is that the technique used in the control groups (cisternal puncture and saline injection) resulted in a certain degree of damage of the blood-CSF barrier as evident by slight CSF pleocytosis in absence of clinical signs of meningitis. Similar CSF reactions have been described after intracisternal administration of blood and its breakdown products to dogs (Jackson, 1949) and after intracisternal administration of PBS (Spector & Lorenzo, 1974).

Meningitic rabbits sacrificed at onset of seizures had markedly higher CSF concentrations of benzylpenicillin than controls. Since the serum concentrations did not differ, this may be explained by inhibition of the transport system responsible for the efflux of benzylpenicillin out of the CSF compartment. That transport system has been shown to be inhibited by several compounds. Moreover, intracisternal inoculation of bacteria to rabbits has also been shown to block this transport system in the choroid plexus, as demonstrated by Spector & Lorenzo (1974). The inhibition of the transport system described by them was similar to the depression of the carrier-mediated glucose transport previously shown by Prockop and Fishman (1968) in canine pneumococcal meningitis. However, glucose transfer has been shown to depend upon a membrane carrier system which has the characteristics of facilitated diffusion (Fishman, 1964b), while benzylpenicillin is actively transported against a concentration gradient (Fishman, 1966; Dixon, Owens & Rall, 1969).

Seizure activity itself is another factor that should be considered as an explanation of the higher CSF and BTF concentrations of benzylpenicillin in animals sacrificed at onset of convulsions. Drug and electrically induced seizures have been shown to produce breakdown of the blood-CNS barriers increasing the penetration of compounds normally restricted from the CNS. This barrier breakdown has been attributed to increased cerebral capillary permeability. Bjerner, Broman & Swensson (1944) and Bauer & Leonhardt (1956) demonstrated penetration of vital dyes into the brain of rabbits after electrically or chemically induced convulsions. Lending, Slobody & Mestern (1959) studied the effect of prolonged convulsions on the blood-CSF barrier of puppies and they could demonstrate a marked increase in the penetration of ^{131}I -albumin into the CSF when seizures were maintained for at least 30 min. Lorenzo et al. (1972) stated that the BBB permeability is temporarily and reversibly increased by seizures; ^{125}I -albumin was cleared from the cat brain after cessation of seizure activity. Since our rabbits were sacrificed

immediately after onset of convulsions, seizure activity seems to be an unlikely explanation for our findings.

As shown in Table V, several differences were noted when comparing the results of this study with those in rabbits with intact blood-CNS barriers (I).

Table V. Comparison between controls with intact blood-CNS barriers (I) and rabbits with experimentally induced E. coli meningitis sacrificed at onset of epileptogenic EEG-activity or seizures. Values are given as mean±SEM.

Criterion	Time to	Total	Benzylpenicillin			Relative	
	criterion	dose	concentration (mg/l)			penetration (%) *	
	(min)	(mg/kg)	Serum	CSF	BTF	CSF	BTF
EEG-changes							
Intact blood-CNS barriers							
	51	550	707	1.28	108	0.18	15.5
	±7	±102	±63	±0.48	±16	±0.05	±1.8
Meningitis							
	66	799	2491	119	214	6.36	9.05
	±14	±220	±334	±49	±25	±3.26	±1.23
Seizures							
Intact blood-CNS barriers							
	103	1767	1282	6.0	243	0.5	19.60
	±21	±566	±134	±1.6	±59	±0.15	±4.9
Meningitis							
	55	748	2077	202	304	8.67	14.28
	±21	±302	±376	±64	±72	±2.54	±2.29

*to concurrent serum concentration

The fact that meningitic rabbits had higher BTF concentrations of benzylpenicillin than those with intact barriers (I) may be explained by some degree of concomitant "breakdown of the blood-brain barrier" in the presence of meningitis as suggested by Bradbury (1979). Supporting this hypothesis are the findings of Bakay (1962) who showed increased brain

levels of tritiated tetracycline in experimental pneumococcal meningitis.

The results of this study strongly indicate that the neurotoxicity of benzylpenicillin is not increased or may even be reduced by meningitis. Thus, at onset of epileptogenic EEG-activity, higher concentrations of benzylpenicillin were found in serum, CSF and brain tissue fluid of meningitic rabbits than in uninfected controls.

Neurotoxicity of Benzylpenicillin in Experimental Renal Failure and Enterobacter cloacae Meningitis (III)

The neurotoxic potential of intravenously administered benzylpenicillin as continuous intravenous infusion at a constant rate (10 mg/kg/min) was studied in rabbits with intact blood-CNS barriers, experimentally established *E. cloacae* meningitis and experimental renal failure secondary to cephaloridine-induced acute tubular necrosis after intravenous administration. Against the background of the marked inter-animal variability in time between onset of epileptogenic EEG-changes and of seizures (I, II), only epileptogenic EEG-activity was used as criterion of neurotoxicity in this study.

The presence of meningitis was confirmed in all infected rabbits as described above (II). Acute tubular necrosis and markedly increased levels of serum creatinine and urea were found in all rabbits pretreated with cephaloridine 48 hr after pretreatment (Table VI). Mean serum albumin levels and haemoglobin were decreased. Anorexia and significant loss of body weight were present in the cephaloridine pretreated groups.

Table VI. Serum creatinine and urea before, 24 hr and 48 hr after cephaloridine pretreatment (100 mg/kg iv).

	Serum creatinine (mmol/l)			Serum urea (mmol/l)		
	Before treatment	After 24 hr	After 48 hr	Before treatment	After 24 hr	After 48 hr
Mean	102	160	377	8.2	10.3	20
SEM±	3	28	62	0.3	0.8	2
N	12	10	10	12	11	11

Table VII gives the concentrations of benzylpenicillin and the doses required at onset of epileptogenic EEG-activity and statistical comparisons between the groups are given in Table VIII.

Table VII. Pretreatment and benzylpenicillin concentrations in serum, CSF and BTF at onset of epileptogenic activity. Results are given as means $\pm$ SEM.

Pre-treatment	Total dose (mg/kg)	Benzylpenicillin concentrations in		
		Serum (mg/l)	CSF* (mg/l)	BTF* (mg/l)
None	886	774	5.4 (0.70)	123 (15.84)
(group 1)	± 113	± 8	± 2.6 (0.34)	11 (1.45)
N	5	5	5	5
Uraemia**	828	1368	5.9 (0.46)	455 (41.23)
(group 2)	± 120	± 258	± 1.5 (0.11)	38 (8.79)
N	6	6	6	6
Meningitis#	473	650	55.1 (10.23)	180 (30.19)
(group 3)	± 184	± 191	± 41.5 (7.68)	± 53 (4.34)
N	4	5	5	5
Uraemia + meningitis	152	622	68.9 (20.90)	187 (36.77)
	± 25	± 139	± 24.4 (11.30)	± 33 (7.29)
N	5	6	6	6
(group 4)				

*Figures within brackets give concentrations as per cent of serum levels. **Cephaloridine 100 mg/kg iv 48 h before experiment.
#Intracisternal inoculation of *E. cloacae* 24 h before experiment.

Table VIII. Statistical comparisons of the treatment groups.

Comparison	Total dose	P values for		
		Benzylpenicillin concentrations in		
		Serum	CSF	BTF
1 vs 2	NS*	NS	NS (NS) #	<0.001 (<0.05)
1 vs 3	NS	NS	NS (NS)	NS (<0.02)
1 vs 4	<0.001	NS	<0.05 (NS)	NS (<0.05)
2 vs 3	NS	NS	NS (NS)	<0.005 (NS)
2 vs 4	<0.001	<0.05	<0.05 (NS)	<0.001 (NS)
3 vs 4	NS	NS	NS (NS)	NS (NS)

*NS, not significant; #Figures within brackets give comparison of concentrations relative to concurrent serum values.

Benzylpenicillin penetrated better to BTF than to CSF in both meningitic and uninfected rabbits. This observation was in accordance with previous findings (I, II). The kinetic differences between the meningitic rabbits in this study and the previous one (II) were probably due to the fact that different bacterial strains were used.

The considerably higher levels of benzylpenicillin assayed in BTF of rabbits with cephaloridine-induced renal failure, when compared to controls with normal renal function, could be explained by several factors related to uraemia. Permeability changes in the blood-CNS barriers have been reported both in experimental uraemic animals and man. Freeman et al. (1962) used the bromide-test as indicator for the integrity of the blood-CSF barrier and demonstrated increased bromide ratio (concentration in CSF/concentration in serum) in uraemic patients, reflecting a generalized dysfunction of the blood-CSF barrier and a derangement of the "overall blood-brain barrier". This abnormality was reversible and disappeared when renal function was normalized. Fishman & Raskin (1967) demonstrated increased brain permeability for radiolabelled inulin and sucrose in rats with uraemia secondary to bilateral nephrectomy, without concomitant signs of brain oedema or increased brain water. In addition, they found changes in sodium and potassium transfer in the brain, suggesting altered activity of the

sodium-potassium ion pump associated with cell membranes. Fishman (1970), using the same experimental model of uraemia in rats, noted inhibition of entry of ^{14}C -penicillin into the rat brain at two hours but not at four hours. He suggested that these findings were due to a competitive inhibition by other organic acids of benzylpenicillin transport in the brain.

Hypoalbuminaemia was one of the consequences of uraemia in the cephaloridine pretreated rabbits. Decreased binding of benzylpenicillin to albumin might therefore have been a factor contributing to the high BTF levels found in uraemic rabbits. Decreased binding capacity of serum proteins in uraemia has been reported (Reidenberg, 1974; Craigh & Suh, 1978b). Other factors possibly contributing to reduced protein binding of drugs in uraemia are conformational changes of the albumin molecule reducing the number of binding sites, or a competition for binding sites by retained metabolites or drugs (Dromgoole, 1974). According to Craig & Suh (1978b), a reduction of the serum protein concentration by at least 50% is required for a doubling of the free fraction of most antimicrobial drugs. Consequently, reduction in protein binding may increase drug distribution to tissues. However, it should be noted that Craigh & Suh (1978b) made these calculations assuming "normal" therapeutic concentrations. We used very high doses of benzylpenicillin, giving serum concentrations several times higher than those achieved during therapy. At such high concentrations, a saturation of binding sites may be achieved and the sensitivity to further reduced albumin levels due to uraemia might increase. It is therefore possible that the free fraction of benzylpenicillin was considerably increased in the uraemic rabbits, thus facilitating penetration over the BBB. Uraemia did not result in increased penetrability of benzylpenicillin into the CSF of non-meningitic uraemic rabbits, which, as expected, increased in rabbits with meningitis alone or with meningitis and uraemia. As previously discussed, this finding can be explained by a lesser affinity of benzylpenicillin for CSF than brain and/or by the active transport of benzylpenicillin out of the CSF space. Inhibition of this transport system together with an increased permeability of the blood-CSF barrier caused by meningeal inflammation may explain the significantly increased penetration of benzylpenicillin into the CSF of meningitic rabbits. Several factors, such as bacterial toxins, inflammatory cell products, CSF acidosis and increased CSF lactate have been suggested as possible inhibitors of this transport system (Spector & Snodgrass, 1974).

As indicated by our results, the blood-brain barrier and the blood-CSF barrier are different entities. Thus, results obtained in CSF can not be extrapolated to brain tissue.

With regard to the neurotoxic potential of benzylpenicillin, the highest brain tissue levels of benzylpenicillin were found in non-meningitic, uraemic rabbits, indicating that the epileptogenic threshold was increased in these animals. The reason why the susceptibility for neurotoxic effects was increased in this model of renal failure remains unclear. The combination of renal failure and meningitis increased the neurotoxicity of benzylpenicillin since the criterion of epileptogenic EEG-activity was reached at lower brain tissue fluid concentrations than in rabbits with uraemia alone. Nevertheless, meningitis, either alone or together with uraemia, did not increase the neurotoxicity in comparison to control rabbits. As previously shown (I, II), higher brain tissue fluid levels of benzylpenicillin were found in meningitic rabbits than those assayed in controls with intact blood-CNS barriers at onset of epileptogenic EEG-changes. Moreover, the pronounced variability of the CSF concentrations found at the criterion in all groups, strongly indicates an absence of a correlation between CSF concentrations and neurotoxicity of benzylpenicillin.

CNS Interaction between Benzylpenicillin and Thiopental (IV)

During the 1960ies the first reports of penicillin induced seizures during cardiopulmonary bypass appeared. Several studies were performed in order to elucidate the factor(s) responsible for the neurotoxic effects. Before that, penicillin had not been administered as infection prophylaxis during open heart surgery. The possible contribution of anaesthesia was not considered in those studies. Although not explicitly stated, thiopental was probably used in the bypass operations. In addition, Bolander & Wahlström (1984) could demonstrate an interaction between probenecid, an inhibitor of the organic acid transport system, and thiopental. Against this background, the effect of benzylpenicillin pretreatment on the kinetics and brain sensitivity for thiopental was studied in rats using an EEG-threshold method previously developed by Wahlström (1966).

Briefly, three experiments were performed. In experiment 1, a group of rats (group A) was preteated with benzylpenicillin ip 50 min before thiopental

(group A) was pretreated with benzylpenicillin ip 50 min before thiopental infusion and EEG-recording. At the EEG-threshold ("the silent-second") they were sacrificed by decapitation in order to interrupt blood flow to the CNS. As controls a group of rats was only given benzylpenicillin (group B) and these rats were sacrificed 50 min later as group A. Another group of rats (group C) was pretreated with saline and infused with thiopental to the silent-second.

Experiment 2 was performed under the same experimental conditions as experiment 1 using a higher dose of benzylpenicillin (1200 mg/kg ip). A group of three rats was given benzylpenicillin and thiopental (group D) and the other group of three rats (group E) was only given benzylpenicillin.

Experiment 3 was performed as an extra control, since the doses of thiopental needed to reach the criterion of silent second after benzylpenicillin pretreatment were found to be very much reduced in group D of experiment 2 in comparison to the saline pretreated rats (Table VIII). This new group (group F) was infused with a fixed dose of thiopental (5 mg/kg) in the same manner as in the threshold determination, but no silent-second was recorded since this dose was too low to induce the EEG-criterion.

The main results of the experiments are given in Table IX, X and XI.

No seizures or other behavioural changes were observed after pretreatment with the lower dose of benzylpenicillin (900 mg/kg), while the higher dose (1200 mg/kg) induced seizures in all rats except one in group E.

The blood content of the assayed brain samples varied between 2.6 and 7.4 $\mu\text{l/g}$ tissue (N=10). This content contributed $0.54 \pm 0.09\%$ (SEM) to the thiopental concentration assayed in brain homogenates and the corresponding figure for benzylpenicillin was $16.6 \pm 2.1\%$. No differences were found between thiopental treated rats and corresponding controls in the blood content of brain specimenes. Since estimates of blood content were not obtained from all animals, brain concentrations were not corrected for blood contribution. However, the blood contribution was too small to significantly affect interpretation of data.

Table IX. Pretreatment and thiopental dose for silent-second. Values are given as means $\pm$ SEM.

Pretreatment	Thiopental dose for "silent-second" (mg/kg)
BPC* 900 mg/kg ip	39.2
(group A)	2.4
N	6
BPC 1200 mg/kg ip	4.9
(group D)	2.2
N	3
Saline	48.3
(group C)	1.9
N	8

*BPC=benzylpenicillin

As shown in Table IX, group A needed considerably lower doses of thiopental to reach the criterion of "silent-second" than group C ($p < 0.001$). The serum concentration of thiopental in group A was also markedly decreased ($p < 0.001$) when compared to controls in group C; however, no differences between these groups were found in the concentrations of thiopental in cortex, hippocampus and brainstem (Table X). Thus, the kinetic process but not the brain sensitivity for thiopental was affected by the lower dose (900 mg/kg) of benzylpenicillin pretreatment.

The brain tissue concentrations of benzylpenicillin were significantly higher ($p < 0.01$) in thiopental treated rats (group D) when compared with corresponding controls in group E (Table XI).

Significantly lower ($p < 0.01$) serum concentrations of thiopental were assayed in group D (pretreated with 1200 mg/kg of benzylpenicillin) when compared with group F which was infused with approximately the same dose of thiopental. However, no differences were found in the brain concentrations of thiopental (Table X).

Table X. Thiopental (T) concentrations in serum, cortex, hippocampus and brainstem in benzylpenicillin (BPC) pretreated rats and controls. Values are given as means $\pm$ SEM.

Group	Treatment*		Thiopental concentration in			
			Serum	Cortex	Hippocampus	Brainstem
	BPC	T	($\mu\text{g/ml}$)	($\mu\text{g/g}$)	($\mu\text{g/g}$)	($\mu\text{g/g}$)
A (N=6)	900	SS#	90.7 $\pm$ 6.0 (59.3 $\pm$ 4.0)	53.2 $\pm$ 3.7	56.9 $\pm$ 4.7	57.7 $\pm$ 3.0
C (N=8)	—	SS	127.6 $\pm$ 5.3 (44.8 $\pm$ 2.1)	56.7 $\pm$ 2.0	54.7 $\pm$ 2.5	60.6 $\pm$ 1.8
D (N=3)	1200	SS	25.5 $\pm$ 6.2 (14.4 $\pm$ 2.5)	3.4 $\pm$ 0.2	3.8 $\pm$ 0.3	4.8 $\pm$ 1.3
F (N=8)	—	5	41.9 $\pm$ 2.0 (8.5 $\pm$ 1.2)	3.5 $\pm$ 0.5	3.3 $\pm$ 0.5	3.8 $\pm$ 0.5

*BPC ip in mg/kg, thiopental dose in mg/kg or dose needed to induce "silent-second" (SS#). Figures within brackets indicate cortex/serum (%).

In order to elucidate the site(s) of the interaction between benzylpenicillin and thiopental, correlations between the studied variables were calculated.

Table XI. Benzylpenicillin (BPC) concentrations in serum and brain tissue of thiopental (T) treated rats and controls. Values are given as means $\pm$ SEM.

Group	Treatment*		Benzylpenicillin concentration in		
	BPC	T	Serum (μ g/ml)	Brain (μ g/g)	Brain/Serum (%)
A (N=6)	900	SS#	666 $\pm$ 21	17.3 $\pm$ 5.7	2.6 $\pm$ 0.8
B (N=8)	900	—	716 $\pm$ 57	15.3 $\pm$ 2.0	2.3 $\pm$ 0.4
D (N=3)	1200	SS	1314 $\pm$ 202	28.7 $\pm$ 2.0	2.3 $\pm$ 0.5
E (N=3)	1200	—	1088 $\pm$ 78	18.3 $\pm$ 0.3	1.7 $\pm$ 0.1

*BPC ip (mg/kg), T=thiopental dose for SS# ("silent-second").

No significant differences were found in the serum and brain tissue concentrations of benzylpenicillin when comparing group A with the corresponding controls in group B (Table XI).

The dose of thiopental needed to reach the criterion was markedly reduced ($p < 0.001$) in rats pretreated with 1200 mg/kg of benzylpenicillin (group D) in comparison with group A pretreated with 900 mg/kg of benzylpenicillin (Table IX).

The serum and brain concentrations of thiopental were considerably lower ($p < 0.001$) in group D pretreated with 1200 mg/kg of benzylpenicillin when compared to controls in group C (Table X).

As shown in Figure 3, a high positive correlation was found between the dose of thiopental needed to reach the criterion of silent-second and thiopental concentration in hippocampus ($R=0.97$, $p < 0.005$) and brainstem ($R=0.91$, $p < 0.02$) in rats pretreated with 900 mg/kg of benzylpenicillin. The corresponding correlations in rats without benzylpenicillin pretreatment were lower (hippocampus $R=0.77$, $p < 0.05$; brainstem $R=0.62$, NS). High negative correlations were found between the dose of thiopental needed to induce the "silent-second" and benzylpenicillin concentrations in brain tissue in both groups pretreated with benzylpenicillin ($R=0.92$, $p < 0.01$ in group A and $R=0.87$, NS in group D). High negative correlations were also found between benzylpenicillin concentrations in brain tissue and thiopental concentrations in hippocampus ($R=0.84$, $p < 0.05$) and brainstem ($R=0.81$, $p < 0.05$) in group A. The corresponding correlations in group D were $R=0.99$ for hippocampus and $R=1.00$ for brainstem but the degree of freedom was only one.

Figure 3. A. Relation between thiopental dose for "silent-second" and concentration of thiopental in hippocampus from rats in group A pretreated with 900 mg/kg of benzylpenicillin (filled triangle) and from rats in group C without benzylpenicillin pretreatment (unfilled triangle). B. Relation between thiopental dose for "silent-second" and concentration of thiopental in brainstem from rats in group A (filled square) and group C (unfilled square).

The high negative correlations between the concentrations of benzylpenicillin and thiopental in brain and the markedly increased brain levels of benzylpenicillin in group D when compared to group E, demonstrate an interaction between the two drugs in the CNS of the rat. A common transport system in the CNS may be the site of this kinetic interaction and competitive inhibition of such a transport system by thiopental might be the mechanism. Several substances are known as inhibitors or depressors of this transport system which is responsible for the active transportation of benzylpenicillin out of the CNS. The competitive inhibition of this transport system by probenecid has been extensively demonstrated (Fishman, 1966; Dacey & Sande, 1974; Spector & Lorenzo, 1974; Walters et al., 1976; Kourtopoulos, Holm & Norrby, 1983).

The differences in thiopental brain/serum quotients between the experimental groups shown in Table X indicate that the distribution of thiopental in the rat brain is not only dependent on lipid solubility but also, as a weak organic acid, on the transport system for organic acids out of the CNS. Support for the hypothesis of a distribution pattern which not only depends on lipid solubility are the findings of Norberg & Wahlström (1986) in their interaction studies on hexobarbital and thiopental. Interactions between probenecid and thiopental have also been shown in experimental animals and man. Bolander & Wahlström (1984) demonstrated increased brain levels of thiopental in probenecid pretreated rats and Kaukinen, Eerola & Ylitalo (1980) showed prolonged thiopental effect after probenecid administration to man.

Lorenzo, Hammerstad & Cutler (1968) and Aquilonius & Winblad (1972) showed that anaesthesia can depress active transport. The fact that the relations found between benzylpenicillin and thiopental in brain were obtained at a fixed level of anaesthesia, excludes the possibility that thiopental acted through its anaesthetic properties.

Seizures have been reported to alter the pharmacokinetics of drugs (Simon, 1980; Monaco et al., 1983). All rats in group D developed seizures. When comparing this group with group A, it had considerably lower brain levels of thiopental at the criterion as well as markedly increased brain levels of benzylpenicillin. Seizure activity itself does not suffice as an explanation for this finding, since Lending, Slobody & Mestern (1959) showed that seizures must last for at least 30 min before an effect becomes apparent and the rats of group D had seizures only lasting a few seconds. Moreover, rats in group E also developed seizures, but the results in this group were different from

those obtained in group D. Thus, seizures can not explain our findings.

Since benzylpenicillin is a CNS excitatory agent, increased doses of thiopental to reach the criterion of silent-second would be expected. However, the reverse was found in group D and no effect on the threshold was seen in group A. It must be concluded that benzylpenicillin as such did not influence the sensitivity of the thiopental threshold in group D, whereas it is possible that the altered brain sensitivity for thiopental may have been induced by seizures.

This study demonstrated a pharmacokinetic interaction between benzylpenicillin and thiopental in the CNS of the rat. The organic acid transport system which both drugs seem to use for transportation out of the CNS, is the most probable site for this interaction. Probably benzylpenicillin and thiopental mutually compete for that transport system.

Benzylpenicillin interaction with anaesthetics could be an important contributing factor in induction of epileptic fits during and after surgery with cardiopulmonary bypass.

Comparative Study of the Neurotoxicity of Benzylpenicillin, Imipenem/Cilastatin and FCE 22101 (V)

The neurotoxic potential of intravenously administered benzylpenicillin, imipenem/cilastatin and FCE 22101 was compared in rabbits. FCE 22101 is a penem derivative, a group of β -lactams with bacterial activity similar to the carbapenems. Both imipenem and FCE 22101 are susceptible to hydrolysis by renal dehydropeptidases. Imipenem is administered with cilastatin to inhibit its renal metabolism by Dehydropeptidase-I (Norrby, 1985). The plasma half-life of each component is approximately one hour. FCE 22101 is less metabolized in the kidneys and is therefore intended for administration without a dehydropeptidase inhibitor.

Neurotoxicity was defined as epileptogenic EEG-activity. The study was performed as a cross-over design with each rabbit as its own control and at least seven days between treatments. No carry-over effects were seen in the rabbits and no detectable antibiotic levels were assayed before a new treatment. Two rabbits had to be excluded; one showed increased irritability and irregular muscle movements without concomitant EEG-changes during

the administration of all three antibiotics (data of this rabbit are not included in the calculations of means since the end-point was not reached). Another rabbit had to be anaesthetized to allow EEG-registration. Therefore, to compensate for loss of evaluable animals, two rabbits received single administrations of FCE 22101 and one was given a single administration of imipenem/cilastatin.

For benzylpenicillin the mean time $\pm$ SEM to the end-point was 47 ± 23 min (range 19-84 min), for imipenem/cilastatin 55 ± 34 min (range 15-127 min) and for FCE 66 ± 27 min (range 12-110 min). Exclusion of data from rabbits which received only one antibiotic administration did not markedly affect the mean values for imipenem/cilastatin or FCE 22101 (56 ± 35 and 62 ± 25 min, respectively).

The main findings are given in Table XII and XIII.

Table XII. Doses and serum concentrations of benzylpenicillin (BPC), imipenem (I) and FCE 22101 (FCE) at onset of epileptogenic EEG-changes. Values are given as mean $\pm$ SEM.

Dose (mg/kg)			Serum concentration (mg/l)		
BPC	I	FCE	BPC	I	FCE
486	86	102	606	55	30
± 84	± 20	± 14	± 100	± 8	± 4
(13)*	(14)	(15)	(13)	(14)	(15)

*Figures within brackets indicate number of rabbits.

Significantly lower doses and serum concentrations of imipenem/cilastatin and FCE 22101 than of benzylpenicillin were required to precipitate epileptogenic EEG-activity. When comparing imipenem/cilastatin and FCE 22101, no significant differences were found for the doses needed to induce the criterion, but the FCE 22101 serum levels were significantly lower than those of imipenem/cilastatin.

Detectable CSF levels of benzylpenicillin were found in all rabbits sacrificed after administration of that antibiotic as the last treatment. After imipenem/cilastatin only one rabbit had detectable levels in the CSF and no rabbit had detectable CSF levels of FCE 22101. In BTF, detectable antibiotic levels were assayed in all tested rabbits.

The highest BTF levels were found after benzylpenicillin treatment ($p < 0.001$ vs imipenem/cilastatin and FCE 22101). No significant differences were noted in the BTF concentrations of imipenem/cilastatin and FCE 22101. However, the relative BTF concentrations (% of the concurrent serum concentrations) were significantly higher for FCE 22101 than imipenem ($p < 0.05$).

Table XIII. Penetration to CSF and BTF of benzylpenicillin, imipenem and FCE 22101 at onset of epileptogenic EEG-activity in rabbits. Values are given as mean $\pm$ SEM.

Parameter	Antibiotic		
	BPC	I	FCE
<u>Absolute concentration</u>			
CSF (mg/l)	2.05 $\pm$ 0.80	NC*	NC
BTF (mg/l)	146 $\pm$ 60	4.7 $\pm$ 1.8	5.5 $\pm$ 1.3
<u>Relative concentration#</u>			
CSF (%)	0.30 $\pm$ 0.01	NC	NC
BTF (%)	18.1 $\pm$ 10.3	9.2 $\pm$ 1.7	16.5 $\pm$ 2.4

*NC, not calculated. #relative to concurrent serum levels.

High correlations were found between the doses administered and the concentrations assayed in serum for benzylpenicillin and imipenem/cilastatin ($R=0.89$, $p < 0.0001$ and $R=0.66$, $p < 0.01$, respectively). For imipenem significant correlations were found between administered dose and BTF concentrations ($R=0.69$, $p < 0.002$) and between serum and BTF concentrations ($R=0.76$, $p < 0.05$).

This study demonstrated that imipenem/cilastatin and FCE are more neurotoxic than benzylpenicillin. It should be noted that both imipenem/cilastatin and FCE 22101 are used or intended to be used at dose levels which are considerably lower than those of benzylpenicillin.

Comparing imipenem/cilastatin and FCE 22101, no significant differences were noted with regard to doses needed to precipitate epileptogenic EEG-activity or BTF concentrations at that time. However, higher serum levels of imipenem than FCE 22101 were assayed at the criterion indicating a more rapid plasma clearance in rabbits of the latter. On the other hand, the lowest relative BTF concentrations were demonstrated for imipenem. An alternative explanation is a better penetration of benzylpenicillin and FCE 22101 than of imipenem across the BBB and/or more rapid clearance of imipenem from brain.

The results obtained with benzylpenicillin were in agreement with previous findings (I, II, III). Our hypothesis that BTF concentrations rather than CSF are decisive for neurotoxicity of β -lactam antibiotics was supported by the fact that detectable CSF levels were only found in one of twelve rabbits treated with imipenem/cilastatin or FCE 22101.

CNS adverse experiences, such as myoclonic activity, confusional states and seizures have been reported with imipenem/cilastatin. Calandra et al. (1988) performed a retrospective study of 1,754 patients treated with imipenem/cilastatin in the United States in order to determine risk factors for seizures. CNS lesions, such as stroke, trauma, tumours, multiple sclerosis, anoxic ischemic encephalopathy, seizure history, renal impairment, excessive imipenem/cilastatin dosage and treatment for *Pseudomonas aeruginosa* infections could be identified as predisposing factors.

Imipenem has been shown to cause seizures when injected intraventricularly to rats (Merck-Frosst, Canada, 1987). At least one metabolite is generated by a non-renal, cilastatin-insensitive dehydropeptidase, which has a prolonged half-life in renal insufficiency (Calandra et al., 1986). This metabolite has been shown to cause seizures when injected intraventricularly to test animals and large doses of cilastatin have also been shown to induce seizures in experimental animals, however the high amount needed to suggest that cilastatin would not be the cause of seizures in the clinical situation (Calandra et al., 1988).

In evaluating the role of parent compounds and the metabolites as causes of neurotoxic reactions to carbapenem and penems, it seems likely that the former are the most neurotoxic. An evidence of that is that rats given high doses of a combination of imipenem/cilastatin and FCE 22101 develop neurotoxic reactions, while imipenem/cilastatin or FCE 22101 alone do not cause such reactions (Farmitalia Carlo Erba, personal communication). Since rats are heavy metabolizers of FCE 22101 (urinary excretion <5% of the dose),

this may be the result of an inhibited metabolism of FCE 22101 by the surplus of cilastatin in the 1:1 mixture of imipenem and cilastatin used. Furthermore, Kahan et al. (personal communication) have demonstrated that in rats, imipenem undergoes systemic lung metabolism in the lung. If this is the case not only for carbapenems, the increased frequency of penicillin neurotoxicity in patients undergoing cardiopulmonary bypass may be due to a reduced metabolism and increased serum concentrations of parent compound. This hypothesis should be tested in patients undergoing cardiac surgery with cardiopulmonary bypass.

The rabbit model used in this study was found to be well suited for cross-over neurotoxicity studies of intravenously administered antibiotics.

Summary and Conclusions

I The EEG-model developed was shown to be a suitable and reproducible method for neurotoxicity studies of β -lactam antibiotics in rabbits. Epileptogenic EEG-activity was a more precise variable than seizures for demonstration of neurotoxicity. BTF levels of benzylpenicillin were very much higher than CSF levels in all rabbits with intact blood-CNS barriers, indicating a better penetration of benzylpenicillin over the BBB than over the blood-CSF barrier and/or a higher affinity of benzylpenicillin for brain tissue. BTF rather than CSF concentrations of benzylpenicillin were decisive for neurotoxicity. The CNS penetration of benzylpenicillin takes place over two different barriers: the BBB and the blood-CSF barrier.

II Experimentally established *E. coli* meningitis, did not increase the neurotoxicity of benzylpenicillin in rabbits. Rabbits with *E. coli* meningitis had higher BTF concentrations of benzylpenicillin than those with intact blood-CNS barriers, indicating some degree of breakdown of the blood-brain barrier. In control rabbits the intracisternal injection of physiological saline resulted in moderate damage of the blood-CSF barrier as evident by discreet CSF pleocytosis in absence of clinical signs of meningitis. Unmanipulated controls are therefore preferable. Meningitic rabbits sacrificed at onset of seizures had markedly higher CSF levels of benzylpenicillin than controls which may be explained by inhibition by bacteria, bacterial toxins or inflammatory cell products of the active transport system responsible for the efflux of benzylpenicillin out of the CSF compartment. The kinetic differences noted between meningitic rabbits inoculated with *E. coli* and those inoculated with *E. cloacae* are probably due to differences in virulence of the two bacterial strains used.

III Uraemia resulted in increased BTF concentrations of benzylpenicillin possibly explained by uraemia-related permeability changes in the BBB and/or decreased binding of benzylpenicillin to albumin. Uraemic non-meningitic rabbits had the highest concentrations of benzylpenicillin at the criterion, indicating that the epileptogenic threshold was increased in these rabbits. Uraemia did not result in increased concentrations of benzylpenicillin in the CSF of non-meningitic rabbits. This can be explained by lesser affinity of benzylpenicillin for CSF than for brain and/or an active transport out of the CSF compartment. Inhibition of this transport system

together with increased permeability of the blood-CSF barrier caused by meningeal inflammation may explain the significantly increased penetration of benzylpenicillin into the CSF of meningitic rabbits. The combination of renal failure and meningitis increased the neurotoxicity of benzylpenicillin, since the criterion was reached at lower BTF concentrations than in rabbits with uraemia alone. Meningitis, either alone or together with uraemia did not increase the neurotoxicity in comparison to control rabbits. At onset of epileptogenic EEG-changes higher BTF levels of benzylpenicillin were found in meningitic rabbits than in controls with intact blood-CNS barriers. A pronounced variability of CSF levels of benzylpenicillin was found in all groups, while the intra-group variations in BTF levels were very much smaller. Thus, BTF and not CSF levels were decisive for the neurotoxicity of benzylpenicillin.

IV Using the "silent-second" as EEG-threshold, a CNS interaction between benzylpenicillin and thiopental was demonstrated in rats. The most probably site for this interaction is the organic acid transport system out of the CNS which both drugs seem to use. Thiopental distribution in the rat brain seemed to depend not only on its lipid-solubility.

V Imipenem/cilastatin and FCE 22101 were shown to be more neurotoxic than benzylpenicillin in rabbits. While BTF levels of the three antibiotics could be detected in all tested rabbits, detectable CSF levels were only found in one of twelve rabbits treated with imipenem/cilastatin or FCE 22101, indicating that BTF concentrations rather than CSF ones are decisive for neurotoxicity of β -lactam antibiotics. The EEG-model used was found to be a suitable model for cross-over studies of intravenously administered antibiotics.

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