Epilepsy, Antiepileptic Drugs, and Birth Defects

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EPILEPSY, ANTIEPILEPTIC DRUGS, AND BIRTH DEFECTS

(Epilepsie, antiepileptica en aangeboren afwijkingen)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
AAN DE ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF. DR. C.J. RIJNVOS
EN VOLGENS HET BESLUIT VAN HET COLLEGE VAN DEKANEN.

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The research described in this thesis has been performed in the department of Cell Biology and Genetics of the Faculty of Medicine and Health Sciences of the Erasmus University Rotterdam, The Netherlands.

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Aan mijn ouders

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PREFACE

Between the covers of this thesis, a cohort of pregnant women with epilepsy receiving antiepileptic drugs, who were referred to the outpatient clinic for prenatal diagnosis of the University Hospital Rotterdam Dijkzigt between November 1985 and July 1990, is desribed.

The objective of this study was to further assess the occurrence of birth defects towards the use of antiepileptic drugs during pregnancy in a prospective manner by ascertainment early in pregnancy, with full attention to clinical details and complete follow-up to three months after birth. Our special interest was to investigate the precise causative relation of valproate exposure and spina bifida, to further delineate the actual risk and to evaluate prenatal diagnosis of valproate-associated neural tube defects.

To place this study in perspective this thesis starts with a review of risks, predisposing factors and postulated mechanisms with regard to the teratogenicity of antiepileptic drugs. Furthermore, an overview of the regulation of amniotic fluid and the disposition of drugs in this compartment will be given as an introduction to these parts of the study dealing with the disposition of valproate and carbamazepine in amniotic fluid. In the last chapter, the results of this study are incorporated in an overview of the recommendations of medical care of epileptic women of childbearing age.

ACKNOWLEDGEMENTS

Many persons have been involved in the project described in this thesis, and the safest and easiest thing to do would be to simply express my thanks to everyone who contributed in so many ways to the realisation of this thesis. Nevertheless, for a personal word of thanks I will take the chance, while keeping to some sort of chronological order.

First of all, I would like to thank Prof. H. Galjaard for the confidence he instilled in me to start this job, as well as for helping me believe I could finish it. Furthermore, I would like to thank all the physicians who kindly referred their patients to our clinic and provided us with essential clinical background information; as well as all the pregnant women with epilepsy for their interest and contribution to this study; the people involved with direct patient care at the outpatient clinic of prenatal diagnosis, the counsellors, those who performed the amniocenteses, the ultrasonographic examinations or the venapunctures. Most of them I have probably never met, but I acknowledge the extra work they did to help this project succeed. Moreover, I would like to thank Prof. E.S. Sachs' well organized laboratory for prenatal diagnosis, which was essential for the success of obtaining complete sampling material. This material in turn reached Margien Witteveen or Henk Janse, who took excellent care of the registration and storage of the material, which involved a tremendous amount of work. I appreciated Prof. E.S. Sachs' personal "pep-talks" very much.

Apart from the several departments who have provided me with the research material, I have worked my way through several of the units of the departments of Cell Biology and Clinical Genetics in Rotterdam, and have been a guest worker in Heemstede and Berlin. Starting out at the laboratory for metabolic diseases at the Sophia Children's Hospital to become acquainted with mass-spectrometry, I would like to acknowledge the personnel with whom I worked in those days, with special reference to Wim Blom and Louis Hierck. I still entertain good memories of the honest conversations which could arise on occasion between the buzzing of the pumps and cooling system. In the same period, I was also a guest worker at the laboratory of the "Instituut voor Epilepsiebestrijding, Meer en Bosch - De Cruquiushoeve", where Jaap Meyer, Nina de Boer, en Dick Vermey introduced me into antiepileptic drug monitoring practice.

After more than a year at Sophia, I changed my working environment to the laboratory of Cell Biology and Histology on the seventh floor of Hoboken, to continue the work initiated at 'Meer en Bosch'. I would like to thank all "inhabitants" there for the social intercourse and the interest they expressed in me and my work. I would like to render special thanks to Andre Hoogeveen, who was always willing to come to the rescue when technical errors occurred, and to Arthur van der Kamp, whose organizing skills were a tremendous help to me. Furthermore, in this regard I would like to thank Piet Hartwijk, apart from the offered welcome distraction, for the engineering of a genious evaporator which allowed considerable speed-up of extraction-procedures.

In 1989, my faith guided me to Berlin to witness the falling of the wall. I have great memories of both the city and the department of Prof. Nau at the Institute of Toxicology and Embryopharmacology of the Free University Berlin, with their regular "sect-parties". Prof. Nau, I am very grateful for your offering me the opportunity to work at your laboratory, for the fruitful discussions and for your critically reviewing the manuscript. Furthermore, I would like to thank Gertrud Beck-Mannagetta for her

hospitality, Erica Drews who helped me to find a place to make my stay comfortable, Werner Wittfoht who helped me out with technical problems, Siegbert, Rosemarie, Thekla and Katherina for sharing their laboratory with me, and Edward Fisher, for both being a wonderful colleague and a dear friend.

After returning from Berlin, the increasing amount of paperwork and telephone calls and the expansion of 'the team', turned the laboratory of Cell Biology more and more into an office and another move was to come. The telephone interviews I had at that time with the young mothers added another valuable human touch to the study, especially in a period when the computer could not be ignored any more. The computer support of Marcel Eijgermans of the Institute of Epidemiology and Biostatistics and Bob Koudenburg, the system operator have prevented me from going nuts, when socalled "computer logic" exceeded mine, and moreover, the enthusiasm of Eric Stassen and Dennis Boon entering the data into the computer made that summer much more enjoyable. With the beginning of fall, Jolanda Groenhuizen helped me to complete the clinical data and together we made the last move to the 24th floor, to the department of Clinical Genetics. The companionship of my 'roommates' Bert de Vries and Senno Verhoef, and our "neighbours" Jolanda van Deursen and Jaqueline du Parant has made the tedious phase of writing this thesis much more pleasant.

Rien Hagenaars of the National Institute of Public Health and Environmental Protection is greatly acknowledged for her contribution to the project, being the determination and interpretation of α-fetoprotein in the maternal sera, as well as reviewing the relevant manuscript.

Ton de Vries Lentsch is acknowledged for drawing the metabolic scheme of valproate and providing me with necessary slides.

Those who have not yet been mentioned in this chronological order of events, are those who remained closely involved over the years. Rick Grobbee, your help at the start of this project with the set up of the protocol, and in the final phase with the epidemiological analysis and writing were essential. The systematic and constructive discussions with you were of indispensable value to complete this thesis. Henk Janse, your continuous care over the obtained biological material has made the analytical part of this study possible. You always provided me with the appropriate samples at the appropriate time, as well as all the other moments when you gave me a helping hand, for which I am very grateful. Frans Los, your continuous dedication to this study became even visible during the statistical analysis, in which it turned out that fewer informed consent were obtained while you were on holiday. Furthermore, I truly appreciate your critically reviewing the manuscripts, which you always accomplished over a weekend.

My expression of thanks ends with the person most directly involved, Prof. Dick Lindhout. Dick, you supported me in so many ways, *e.g.* when things became more and more medical you helped me to judge and classify, and you have taught me how to write scientific papers. Especially, under changing conditions, we have shown the same interest and attitude of perseverance to complete this research project as well as possible. I truly appreciate the many discussions we had, and I admire your method of argumentation. Moreover, I am very grateful to you for giving me the freedom and time to follow educational programs, other than the Erasmus curriculum.

Finally, the love, reassurance and encouragement of my parents, brothers and sister, and dearest friends were essential for the preservation of happiness.

CHAPTER 1

INTRODUCTION

EPILEPSY, ANTIEPILEPTIC DRUGS AND BIRTH DEFECTS A REVIEW

1.1.1 Introduction

Up to the beginning of this century the treatment of an epileptic patient was limited to lifestyle adjustments, like regularity and certain diet restrictions (*i.e.* no meat), with bromides as the only available medication. These bromides worked mainly through sedation. With the availability of phenobarbital (1912) and phenytoin (1938) for the treatment of epilepsy a considerable progress was initiated, and this continued by the introduction of other less sedative antiepileptic drugs, like valproate (1964, Europe) and carbamazepine (1961, France). This improvement in the treatment of epilepsy, combined with more tolerant attitudes in society, led to a better social adjustment and allowed more patients with epilepsy to live normal lives.

The thalidomide disaster in the early sixties was the starting point of an increasing awareness of the potential hazards of chemical agents to the unborn child. Since epilepsy is a rather common disease and most women of childbearing age with epilepsy are chronically treated with antiepileptic drugs, the teratogenic potential of this group of drugs has attracted extensive attention.

Despite the well documented 2 to 3 fold increase in risk for the occurrence of major congenital malformations in the offspring of treated epileptic women as compared to the general population as well as to non-treated epileptic women, there is general agreement that epileptic treatment should be continued during pregnancy in most patients (1-3). Several reasons can be given for this apparent inconsequence.

First, a large proportion of women with epilepsy will simply become pregnant while on antiepileptic drugs. A subsequent slow dose reduction, required for safe discontinuation of antiepileptic drugs, after recognition of pregnancy a few weeks after conception will not prevent the embryo from exposure during the critical period of organogenesis.

Secondly, in patients with active epilepsy, the risk of seizures outweighs the risk of antiepileptic drug induced teratogenesis. Continuation of treatment is, in general, regarded a necessity (1-3) because of the risk of trauma associated with seizures for the

woman herself as well as the fetus. Status epilepticus has a high maternal and perinatal mortality (4), whereas other possible adverse effects of maternal seizures on the developing fetus might be hypoxia, observed as hypoxic signs in fetal heart rate (5), lactic acidosis (6), and altered neuroendrocrine function (7). It has been suggested that seizure-induced hypoxia and acidosis in the first trimester of pregnancy could cause structural defects or when they occur in the third trimester may lead to cerebral palsy and mental retardation (3).

Besides, controversy remains about the teratogenicity of antiepileptic drugs *per se*. Although the increased risk of malformed offspring for treated epileptic mothers as compared to untreated epileptic mothers suggests a specific role for antiepileptic drugs in the etiology of malformations, the observed difference may be confounded by a most likely difference in type and severity of epilepsy between treated and untreated women. It remains extremely difficult to distinguish between the treatment and the underlying illness. Thereby, since many birth defects are inherited as multifactorial polygenetic traits, prior to accusing antiepileptic drugs of teratogenicity it is mandatory to explore whether other (co)factors, associated with the maternal epilepsy or of obstetrical or sociological nature, in part or alone, are responsible for the increased anomaly rates in the progeny of treated epileptic mothers. Drug specificity of malformations and elucidation of mechanisms of teratogenesis by antiepileptic drugs would help to evaluate the precise etiological role.

1.1.2 Major malformations

In the past 30 years, more than about 70 epidemiologic studies have been concerned with the association between exposure to antiepileptic drugs *in utero*, and an elevated risk for both major and minor congenital malformations as well as abnormalities of growth and development (8, 10). The first formal investigation of this kind dates from 1964 (11), after the teratogenic effects of thalidomide had become evident.

The vast majority of the epidemiologic studies which compared treated epileptic mothers with the general background population showed a 2 to 3 fold increased risk of congenital malformations for the offspring of epileptic mothers.

Different approaches have been used, both retrospective (12-21) and prospective cohort studies (21-28) as well as some case-control studies (29, 30), with differences in ascertainment of study populations (varying from birth registries, neurologic and obstetric clinics, to specialized epilepsy clinics), different definitions of major and minor malformations, follow-up period etc. Possible consequences of these differences will be discussed below under methodological considerations.

By comparing treated epileptic women with the general background population, factors associated with the maternal epilepsy can not be evaluated separately. For this purpose comparisons have been made between treated and untreated epileptic women. With a few exceptions (27, 29, 32-35), the vast majority of these studies (11, 13-19, 26, 36-40) show a 2 to 3 fold increased prevalence rate among the drug-treated group.

Thereby, the prevalence rate of major malformations in offspring of untreated epileptic patients was generally not (13-18), or only slightly (19, 27) increased compared to the general population. These results suggest strongly an etiologic role for antiepileptic drugs in dysmorphogenesis. Nevertheless, possible confounders in this kind of studies might be differences in the underlying epilepsy between those requiring treatment and those who do not.

Medication specific risks;
Differences between polytherapy and monotherapy, and between different single drugs.

As already mentioned above, in most women treatment remains necessary during pregnancy, and therefore the clinically important question is which specific antiepileptic therapy bears the lowest teratogenic potential. Besides, if type and risk of malformations significantly differ with different therapies, a causal relationship is more likely.

Comparisons between monotherapy and polytherapy have been consistent in reporting an up to 2 times higher risk with increasing numbers of antiepileptic drugs used concomitantly (11, 16, 19, 23, 32, 36, 37, 40-43).

But apart from this difference, the available published data are insufficient to demonstrate statistically significant differences in risk among commonly-used monotherapies. Due to the frequent use of polytherapy, especially in the past, the numbers on specific single drug exposures are still limited. Furthermore, to make analysis possible within a study, most of them have presented only denominators for any exposure to a particular drug, not breaking the total down to the drug used alone or in combination to other drugs, by which, however, specific information on monotherapy has been lost. Moreover, in about three quarters of the reported pregnancies phenobarbital and/or phenytoin were used, both among the monotherapies as well as in polytherapy. Data on carbamazepine and valproate exposure are not so extensive, yet. Comparisons between monotherapies made with the currently available data suggest slightly lower teratogenic risks for phenobarbital and carbamazepine than for phenytoin and valproic acid (15, 16, 18, 19, 23, 36, 42-44).

Apart from the limited statistical power due to limited numbers, the problem to distinguish might also be explained by comparable teratogenicity of different antiepileptic drugs or by a considerable influence of constitutional factors.

Specific types of major congenital malformations

Of the malformations observed in treated epileptic patients, facial clefts and congenital heart defects are the most frequent, each in about 2% of births to epileptic mothers (21). Many studies have been concerned with these defects, in particular. Several case-control studies have been conducted with regard to facial clefts, whereas systematic case-control studies on congenital heart defects are much more limited, probably due to

methodological problems caused by the heterogeneity of the defect and the fact that the diagnosis or onset of symptoms of heart defects is health-care system and age dependent.

Both defects are more frequently observed than expected with phenobarbital (44-48) and phenytoin (44-49) exposure, whereas for facial clefts, in several studies a significant association was observed with diazepam as well (48, 50-52), but mainly used as part of combination therapy.

Compilations over different prospective and retrospective cohort studies have been made (10, 53-55) for both facial clefts and congenital heart defects. The relative risk for these specific defects is considerably higher than the overall increased risk of 2 to 3 fold, with estimates for facial clefting of 9 to 13 fold based on cohort studies, and for congenital heart defects 2 to 4 fold based on cohort studies (for review see 10). Since cleft lip with or without cleft palate and isolated cleft palate are etiologically distinct (56), a further detailed analysis showed that in antiepileptic drugs exposed infants a preponderance of cleft lip with or without cleft palate was observed as compared to the general population (10), but that the risk of isolated cleft palate was only moderately increased (*i.e.* 3 fold).

More recently, evidence for a 10 to 20 fold increased risk compared with the general population has been reported for neural tube defects in association with valproic acid and carbamazepine exposure. This evidence comes from both case-control studies as well as collaborative prospective studies. Almost 10 years after the introduction on the European market, the first expressions of concern about the possible teratogenic effects observed with valproate in mice, including neural tube defects, appeared in the literature (57, 58). Following this warning, Gomez (59) reported a child with a lumbosacral meningocele, after first trimester valproate exposure. In 1982, Robert and Guibaud (60) reported data from the Rhône-Alpes birth defects register covering a 3 years period. Among 72 infants with lumbosacral neural tube defects, 9 cases had been exposed to valproate *in utero*. Three of them also had a relative with spina bifida. Subsequent reports, partly from other groups, supported the teratogenic potential of valproic acid (29, 61-63), but discussion continued on methodological issues as well as on the causality of this relationship. To overcome some of the methodological problems and limited sample sizes, resulting in very wide 95% confidence limits (CI), large international collaborative studies were desirable. The combined results of 13 out of 18 independent prospective study groups on the teratogenicity of antiepileptic drugs showed a frequency of neural tube defects after valproate exposure of 1.5% (95% CI: 0.3% to 2.7%). More specifically, the risk for infants exposed to valproate monotherapy was 2.5% (95% CI: 0% to 5.3%), and 1.1% (95% CI: 0% to 2.3%) when valproate was used in combination with other antiepileptic drugs. In this study, the risk for infants exposed to other antiepileptic drugs than valproate was 0.35% (95% CI: 0.1% to 0.6%). This estimate for other antiepileptic drugs, was similar to the risk of neural tube defects in treated epileptic patients before the introduction of valproate (64).

Several clinically important questions remained to be answered. The severity as well as the nature of the defect are important. Current knowledge suggests a very similar etiology for anencephaly and spina bifida (65). Valproic acid, however, appeared to be

specifically associated with spina bifida in humans, but selective ascertainment could also be an explanation for this observed specificity. Animal experiments have further suggested that both dose and administration schedule are important in the increased valproate-induced exencephaly risk in mice (66), whereas human data on dose-relationship are still missing.

Soon after the first reports of an association between valproate and spina bifida, more spina bifida cases were also reported in relation to carbamazepine exposure (61, 67). Recently the risk for carbamazepine, without concurrent exposure to valproate, was estimated at 0.9%, based on combined data from birth defects registries as well as cohort studies. Within this pooled data set the relative risk with carbamazepine as compared to valproic acid was 0.6 (95% CI; 0.2 to 1.7) (68).

Prenatal diagnosis of neural tube defects is possible. The risk associated with valproate and carbamazepine is 10 to 20 fold that observed in the general population, which is similar to the risk of parents with a previous child with a neural tube defect. Therefore, prenatal diagnosis was offered to women taking valproate and carbamazepine from 1984 onwards (61), at least in The Netherlands.

The detection of the potential of valproate to induce neural tube defects has clearly demonstrated the success of the birth defects registries which have been established in many parts of the world, often in response to the thalidomide tragedy. However, it also reveals the need for large additional international prospective studies (or even surveillance systems, reporting to a general scientific board), which should include detailed clinical information of possible confounders associated with the underlying disease, as well as family data, to counter discussions on methodological limitations which has caused a delay of several years before general acceptance of a causal relation between valproate and spina bifida was a fact. This is especially important for obtaining a rapid evaluation of the teratogenicity of newly introduced antiepileptic drugs, such as already can occur accidentally in ongoing clinical trials.

1.1.3 Inherited susceptibility to malformations

Most congenital malformations are considered to be from a multifactorial origin, determined by both genetic and environmental factors (69). One of the principles of teratogenesis formulated by Wilson (70) is that a teratogen must operate on a susceptible genotype, which ultimately influences its phenotypic expression (table 1.1). Thus, the concept of a genetic predisposition (53) whether associated with epilepsy or not, can not be neglected. Many case reports have been published on families with more than one affected child born after the mother had used the same antiepileptic medication during successive pregnancies (71-74), also suggesting the involvement of genetic factors. Moreover, higher prevalence of malformations in relatives of offspring with major malformations as compared to that for unaffected children have been reported in several studies (12, 18, 19, 32, 37, 47, 75).

Table 1.1 Six fundamental principles of teratology, according to Wilson, 1977 (69)

1. Susceptibility to teratogenesis depends on the genotype of the conceptus and the manner in which this interacts with environmental factors.
2. Susceptibility to teratogenic agents varies with the developmental stage at the time of exposure.
3. Teratogenic agents act in specific ways (mechanisms) on developing cells and tissues to initiate abnormal embryogenesis (pathogenesis).
4. The final manifestations of abnormal development are death, malformation, growth retardation, and functional disorder.
5. The access of adverse environmental influences to developing tissues depends on the nature of the influences (agent).
6. Manifestations of deviant development increase in degree as dosage increases from the no-effect to totally lethal level.

Genetic predisposition, common to malformations and epilepsy?

Although epilepsy might be a nonspecific symptom of various cerebral disorders, a number of specific genetic entities can be identified (76). Since both, epilepsies and major congenital malformations are known to be inherited as multifactorial polygenetic traits involving the interaction of many genes with environmental factors, it is possible that these two groups of disorders may share some genes in common (27, 53).

One approach to study this hypothesis has been by comparing treated and non-treated epileptic women, which as mentioned above, shows an increased risk for the treated group, whereas the risk for non-treated women did usually not exceed that of the general population.

If genetic factors associated with epilepsy are involved in the etiology of congenital anomalies in the progeny of epileptic patients, then one would expect an elevated incidence of these malformations in offspring of epileptic patients of *both* sexes. However, the reported data on this comparison are inconsistent. Whereas some studies found higher anomaly rates for epileptic women, with those for men not exceeding that of the general population (19, 27, 36, 39, 77), other studies reported a slightly higher frequency of major malformations in the offspring of epileptic men as compared with those for epileptic women or controls (38, 40, 78). Results might be confounded by differences in reproductive fitness between epileptic mothers and fathers. Rates of reproduction have been found lower in epileptic men than epileptic women (18, 79, 80) and Dansky *et al.* (79) suggested that males who reproduce may have less severe epilepsy than do epileptic women who reproduce. Moreover, if higher malformation rates in children of epileptic fathers are found, the possible effect of antiepileptic drugs on chromosomal rearrangement or mutations in the paternal genome should be ruled out.

Mutagenic effects have been described in various test systems *in vitro* (81-84). Increased chromosomal damage and sister chromatid exchanges in peripheral blood lymphocytes of patients receiving chronic antiepileptic treatment have also been observed (85-87). De novo gene mutations in association with antiepileptic drug exposure *in utero*

have been suggested as well as transplacental carcinogenesis in case reports of embryonal tumors, like Wilms tumors, malignant mesenchyoma, and ganglio-neuroblastoma, in association with prenatal phenytoin en carbamazepine exposure (88, 89). The extrapolation of the, sometimes contradictory, *in vitro* data, remains difficult. Moreover, the interpretation of the limited human observations are complicated by the heterogeneity of the tumors, the relative low frequency and the lack of well defined exposure denominators for these single case reports. The mutagenic potential of chronic antiepileptic drug treatment and its role in teratogenesis remains a subject for further study, in which of course also the parents and family should be evaluated to exclude mere inheritance of the defect.

Genetic predisposition, common to facial clefts and epilepsy?

Since facial clefts are among the most frequently observed malformations in the progeny of epileptic parents, several studies have been concerned with a possible association of the liability of developing epilepsy and facial clefts, in particular. Cleft lip with or without cleft palate is inherited as a multifactorial polygenic trait, with equal rates of about 3 to 4% in sibs and children (*i.e.* first degree relatives) of facial cleft probands, which is about 40 times the rate in the background population (90, 91).

Thus, a genetic association would be likely when an increased facial cleft prevalence in the epileptic parents themselves would be observed or moreover when the frequency of facial clefts is equally increased in both sibs and children of probands with epilepsy. An increased facial cleft rate, only in children of treated mothers, would on the other hand, favour an etiological role of the antiepileptic treatment, unless clefts associated with epilepsy represent a subgroup of clefts with a mode of inheritance other than polygenic.

Only one study has investigated the prevalence of orofacial clefting among epileptic patients themselves (92). Cross-matching of records of 3203 unselected probands with epilepsy with a complete file of all Danish facial cleft patients (n=5756, born between 1934-1977), showed a prevalence of facial clefts in the epileptic population twice as high as expected (11 *vs.* 5.1, $p<0.05$), with 7 male and 4 female patients, with no signs of preponderance of any specific type of cleft or epilepsy. This could be due to two closely linked genes (for epilepsy and clefting) which might be transmitted together more frequently than by chance alone. However, a possible bias may be introduced since patients were unselected and therefore we can not be certain whether some patients had epilepsy and clefting as part of a syndrome associated with chromosomal abnormalities, which could lead to an overestimation of the association between epilepsy and non-syndromic clefting.

Subsequent investigations of this study group involved the prevalence of facial clefts in sibs and children of patients with epilepsy (93). In this study the prevalence of facial clefts in sibs and children of 2072 patients with epilepsy was ascertained, again by a questionnaire and record linkage with the same Danish national facial cleft file. The

prevalence of orofacial clefts in children of fathers with epilepsy (one isolated cleft palate) and in siblings of epileptic probands was not increased, but observed rates were significantly higher than expected when the mother had manifested epilepsy, with 3 cleft lips with or without cleft palates observed among exposed infants (observed/expected ratio; 3/0.633, 4.7) and one among untreated mothers (observed/expected ratio; 1/0.414, 2.4). The prevalence of isolated cleft palate was not above expectation in any of the groups of relatives. These results favour a predominant role for antiepileptic drug exposure. Besides, the low orofacial cleft prevalence in these first-degree (*i.e.* sibs and children) relatives of probands with epilepsy as compared to first degree relatives of facial cleft probands (*i.e.* 5 times *vs.* about 40 times the prevalence in the general population) also suggests that an association between epilepsy and cleft lip with or without cleft palate is unlikely.

Approaching the subject from the other side; an increased epilepsy prevalence among parents and other close relatives of facial cleft probands would be expected. In early studies conducted according to this approach, such an increased incidence of first- and second-degree (94) (and third-degrees in reference 95) relatives with epilepsy was reported for patients with cleft lip with or without cleft palate. Similar epilepsy prevalences of 2.3% for mothers and 1.8% for fathers among parents of facial cleft children (96), with background rates of 0.75%, also suggested that facial clefts in general and epilepsy could be associated genetically. However, when cleft lip with or without cleft palate and isolated cleft palate were considered separately, a preponderance of cleft lip with or without cleft palate was noted among children of mothers as opposed to fathers with epilepsy, *i.e.* 9 *vs.* 2, whereas for isolated cleft palate the score was 2 mothers *vs.* 5 fathers. Combining data from these studies showed that only cleft palate was increased in the offspring of epileptic fathers (0.14%, which is about twice that found in the general population (10).

Thus, these epidemiologic studies do not provide conclusive evidence for a genetic aggregation of clefts and epilepsy. A different approach using molecular genetic methods might help to answer this question in the future. For example, both genetic studies in families of patients with cleft lip with or without cleft palate, and genetic studies in families of patients with epilepsy (*i.e.* juvenile myoclonic epilepsy), show evidence for the possible existence of genes on the short arm of chromosome 6. Whereas the suspected gene for orofacial clefting is linked to factor XIIIa, the gene for idiopathic generalized epilepsy is close to the HLA region. Both genes are not identical, and are probably not closely linked (for review see 97). This suggests no direct familial aggregation of orofacial clefting and this type of epilepsy, but further research along this line might increase our knowledge of genetic factors influencing expression of epilepsy and developmental disturbances.

Genetic predisposition, common to congenital heart disease and epilepsy?

Isolated congenital defects of the heart and great vessels include a wide variety of different anomalies, with different frequencies, sex distributions and recurrence risks, which might partly be due to genetic diseases or various environmental factors. Thereby, heart defects might frequently occur in specific syndromes as well as a number of chromosomal aberrations. Besides, whereas orofacial clefts are easily observed at birth, the possibility of incomplete ascertainment is much larger for congenital heart disease. Some cases without symptoms or signs may go unnoticed at birth, and underascertainment of lethal cases might occur also, especially in a questionnaire /record linkage study. This has made it even harder to investigate a possible association between epilepsy and congenital heart disease than between epilepsy and clefts.

An initial report of Kelly *et al.* (95) suggested an association between orofacial cleft, congenital heart defects and/or epilepsy. Of 162 probands with cleft lip with or without cleft palate, and without parental epilepsy, 17% and 16%, respectively, had a family history of epilepsy and of congenital heart defects. Isolated cleft palate did not appear to be associated with congenital heart defects or epilepsy. Another report (98) found a slight excess in the parents of women with epilepsy, but not in other relatives.

In a more elaborate study of Friis and Hauge (99) among 2461 children of epileptic parents, 18 cases of congenital heart defects were observed (8/1092, 0.75% with epileptic fathers and 10/1399, 0.71% with epileptic mothers), with background population values of 0.80%. Thus, in this study neither a genetic association between epilepsy and congenital heart defects, nor teratogenicity of treatment was observed.

In conclusion, although genetic factors are important in the occurrence of congenital malformations, studies concerned with possible genetic associations with epilepsy have not been able to demonstrate a significant aggregation.

Genetic predisposition, common to neural tube defects and epilepsy?

As stated already for orofacial clefting and congenital heart defects, also neural tube defects can occur in different forms, from anencephaly to various types of spinal dysraphism, presented alone or as part of a syndrome. Neural tube defects tend to cluster within families, and several modes of transmission have been regarded, but no clear single association between (non)genetic factors has been established thus far (100).

Recently, Klepel and Freitag (101) reported that spina bifida occulta in the lumbar and/or sacral region was almost twice as common (25/84, 30%) in patients with idiopathic epilepsy as compared to patients with symptomatic epilepsy (19/98, 19%) or the general population (17%). Although this difference is not significant, it prompts future research in this direction. For example, it would be interesting to see if parents of a fetus or child with spina bifida aperta after valproate exposure show a higher frequency of spina bifida occulta. Radiographic studies of the lumbar vertebral column and sacral region of valproate exposed infants would also be of interest with regard to a possibly

higher frequency of spina bifida occulta. Especially, since it has been demonstrated in mice that with appropriate timing of valproate administration, a low frequency of spina bifida aperta could be induced among a high frequency of the occult form (102).

Genetic predisposition to drug-induced malformations?

Pharmacokinetic differences in the metabolism of antiepileptic drugs might have implications with respect to the extent of fetal drug exposure and subsequently with the occurrence of possibly drug-induced birth defects.

Differences in plasma concentrations of the ultimate teratogenic agent, being the drug itself or one or more of its metabolites, are determined by dose and individual differences in absorption, weight, volume of distribution, metabolism and clearance. Apart from genetic differences, variations in metabolism might also be introduced by other environmental factors, like the diet, comedication, or other exposures to inducing or inhibiting agents. Pregnancy itself causes several physiological changes which might lead to alterations in pharmacokinetics.

Placental transfer has been demonstrated for all antiepileptic drugs with concentrations in first trimester umbilical cord plasma ranging from 40 to 80% of those measured in maternal plasma (103). Within the fetus, phenytoin (104), phenobarbital (105) and carbamazepine (106) tend to accumulate in the fetal brain, liver, adrenal gland and kidney. Thereby phenytoin has been shown to accumulate in the fetal heart (104). For valproate human data are not available, but embryonic accumulation has been demonstrated in the neuroepithelium of the mouse during early organogenesis (107).

The human embryo and fetus is capable of some drug metabolism, but in general the enzyme activity is low compared with adult levels (108). It has been postulated that reactive intermediates produced during antiepileptic drug metabolism, by the mother or fetus or both, might be responsible for fetotoxicity by binding to fetal macromolecules or nucleic acids.

Differences in enzyme activities resulting in increased amounts of reactive intermediates could be the result of pharmacogenetic differences (109, 110), or due to metabolic interactions between different antiepileptic drugs (111). This last hypothesis is also supported by the concomitant reduction in malformation rates with the increasing replacement of polytherapy by monotherapy over the past years (40, 112). Experimental studies on these topics will be discussed in more detail in relation to possible mechanisms.

Human lymphocytes and amniocytes have been used in *in vitro* assays to study possible genetic differences in sensitivity towards teratogenic effects of antiepileptic drugs, but results are preliminary and inconsistent. With lymphocytes obtained from children gestationally exposed to phenytoin, the observed toxicity (*i.e.* cell death) to phenytoin metabolites generated by murine hepatic microsomes *in vitro*, correlated with the occurrence of major birth defects in these children, yet not with minor anomalies nor with growth parameters at birth (108). They speculated that the observed difference in

cell death was the result of a detoxification defect, for which family studies suggested an autosomal codominant inheritance.

Buehler and coworkers (109) performed activity measurements of epoxide hydrolase, one of the enzymes involved in the detoxification of arene oxides, in amniocytes, obtained from exposed pregnancies. The apparently trimodal distribution of the enzyme activities they found in the amniocytes of control fetuses, suggested that this epoxide hydrolase was regulated by a single gene with two alleles, which would make fetuses homozygous for the recessive allele, *i.e.* with low epoxide hydrolase activities, more at risk for the clinical features of the fetal phenytoin syndrome. Nevertheless, other studies have reported an unimodal distribution in epoxide hydrolase activity in lymphocytes of a control population (113, 114). Moreover, different iso-enzymes of epoxide hydrolase exist with different substrate specificities, subcellular localization and expression patterns in various tissues, most likely encoded by a family of genes (115).

Of the 19 prospectively monitored pregnancies with phenytoin monotherapy, low enzyme activities (<30% of the standard) in amniocytes were observed in 4 cases. After births those 4 infants had clinical findings compatible with the fetal phenytoin syndrome. The authors suggested that this enzymatic biomarker could predict the occurrence of minor anomalies. These preliminary results could support the concept of genetic susceptibility, but larger numbers are required to confirm these observations. Before a mode of inheritance can be determined family studies should be performed, including exposed, but unaffected siblings. Furthermore, the possibility of a permanent change - persisting into postnatal life - in expression of epoxide hydrolase activity due to prenatal phenytoin exposure, which might coincided with its teratogenic effects, should also be ruled out (116, 117).

1.1.4 The possible adverse effects of fits during pregnancy

Although the possibility of fetal damage from epileptic seizures has been raised frequently, only seldom this risk factor has been included in the investigations. However, most large-scale studies have found no evidence for an etiological role of epileptic seizures during pregnancy in the occurrence of malformations in the offspring (22, 37, 76). One experimental study has been conducted to separate the role of the seizure from that of the medication on adverse pregnancy outcome (118). When inbred mice homozygous for a genetically determined spontaneous seizure disorder known as quaking (*qk/qk*) were left untreated throughout gestation they produced normal healthy pups. In homozygous quaking (*qk/qk*) dams treated with increasing doses of phenytoin, the frequency of seizures decreased while the incidence of congenital malformations increased. Although malformation rates do not appear to be influenced by fit frequency, epileptic seizures, especially single or repeated severe generalized tonic-clonic attacks with hypoxia and metabolic changes such as acidosis (6, 7), bear the possibility of abortion or fetal death.

1.1.5 Other possible risk factors

Since factors such as an increased maternal age or a low socioeconomic class are known risk factors for the occurrence of congenital anomalies in the general population, these factors have been included in several studies, but without strong evidence to be of major importance on the observed increased incidence of major malformations in the progeny of women with epilepsy, receiving antiepileptic drugs. Folate deficiency, due to dietary insufficiency and/or induced by antiepileptic drug treatment may be a additional risk factor. This folate deficiency has been proposed as one of the possible mechanisms of the teratogenic action of antiepileptic drugs and will be discussed in paragraph 1.2.

1.1.6 Minor anomalies

Besides the increased occurrence of major congenital anomalies, several dysmorphic features appear to occur more often if the mother is treated for her epilepsy during pregnancy. The occurrence of minor anomalies in the offspring of epileptic women was already investigated in 1964 by Marden and coworkers (119). They used as definition for minor anomalies: A minor anomaly is a physical defect that occurs in fewer than 4% of infants, whereas the term "normal phenotypic variant" was used for more common physical defects. Although most investigators have used this distinction, the variable definitions and observations used to diagnose a particular "anomaly" or "phenotypic variant" have led to widely variable results among studies.

The interest in these minor anomalies might be explained partly by the apparent medical significance suggested by the finding that those children exposed to phenytoin *in utero* who had a consistent pattern of craniofacial abnormalities, including shortened cranial base and maxillary hypoplasia, had a significant chance of having cognitive dysfunction (120).

Many reports have described various dysmorphic craniofacial features in association with antiepileptic drugs exposure during pregnancy, including hypertelorism, inner-epicanthal folds, eye slant, ptosis, strabismus, flat nasal bridge, low set ears, and abnormalities of the extremities that include hypoplasia of the nails and distal phalanges and changes in dermatoglyphics.

Several researchers have tried to fit the major malformations and minor dysmorphic features into distinct syndromes, such as the trimethadione syndrome (121, 122), the fetal hydantoin syndrome or phenytoin syndrome (123), the barbiturate syndrome (124) and the fetal valproate syndrome (125, 126). However, none of these syndromes seems very drug-specific, and also the incidence after exposure varies considerably between studies, ranging from 6 to 46%, with an average of probably less than 10% (8, 11). This has led others to propose a general anti-epileptic drug-syndrome, consisting of congenital heart defects, orofacial clefts, and trigonocephaly or microcephaly as major abnormalities and various minor anomalies including hypertelorism, low set abnormal ears, short neck with low posterior hairline, bilateral single transverse palmar

creases and minor skeletal defects, especially of the distal phalanges. Furthermore, mental subnormality may also be a feature of the syndrome. The syndrome might be highly variably expressed, with individual features occurring alone or in combination or in association with prenatal and/or postnatal growth deficiency.

The trimethadione syndrome is probably one of the best established with the prevalence among prenatally exposed infants. This syndrome includes congenital heart defects and developmental delay, besides more specific craniofacial anomalies, like V-shaped eye-brows (121, 122).

The fetal phenytoin syndrome (123) is already somewhat more controversial, of which the most frequently observed abnormality is the variable degree of hypoplasia and irregular ossification of the distal phalanges of both fingers and toes. Microcephaly and mental retardation are also often reported. Craniofacial features include broad nasal bridge, short upturned nose, hypertelorism, epicanthal folds, and ptosis, but these craniofacial features have also been reported after other antiepileptic drug exposures, like valproate (126), or even genetically linked to epilepsy (53, 127).

The described fetal valproate syndrome (125, 126) includes major anomalies of the central nervous system, such as neural tube defects, the skeleton including aplasia of radius and rib anomalies, and the urogenital system, like hypospadias. Thereby minor anomalies are reported, such as brachycephaly, high forehead, shallow orbits, flat nasal bridge, small nose, hypertelorism, long upper lip with shallow philtrum, and low set and rotated ears.

Recently, Jones and colleagues (128) reported that carbamazepine was not so much associated with an increased risk for major birth defects, but especially associated with an moderate increase of minor craniofacial defects, fingernail hypoplasia and developmental delay. The similarities observed between carbamazepine and phenytoin exposed infants did them suggest that epoxide intermediates of carbamazepine rather than the parent drug could be the ultimate teratogen, since both drugs are metabolized through arene oxide pathways. However, the observers were far from blind for the exposure. All cases were exposed to carbamazepine, either alone or in combination with other antiepileptic drugs. Unfortunately, parental features were not evaluated and children from non-epileptic women were used as controls in the evaluation of the minor anomalies. The possibility that the minor anomalies are part of a syndrome genetically related to epilepsy is thereby neglected. Furthermore, they reported a 20% (5/25) incidence of developmental delay among the exposed infants, which they based on observed scores of 1SD or more below the mean on developmental tests. Since 16% of an adequate sample of a population with a normal distribution will fall below the limit of 1SD of the mean, the reported 20% incidence does not significantly differ from the predicted normal population.

Most of the evidence of minor anomalies comes from case reports or small prospective studies, with potential observer bias, due to the observer being not blind for the exposure. Only one larger (108 exposed *vs.* 108 matched control children examined at the age of 5.5 years) and methodologically more refined study was conducted in Finland (127). The observers were blind for the exposure and multiple logistic regression analysis was used to adjust for genetic factors linked to the epileptic condition. They found that

39% of the offspring of epileptic mothers had two or more minor anomalies, considered typical for the phenytoin syndrome, as compared to 15% of the controls. They found also that epicanthus, low hairline, nail hypoplasia and 3 or more dermal arches were significantly associated with the presence of these features in the mother. Only hypertelorism and digital hypoplasia appeared to be significantly associated with phenytoin exposure. More evidence for the importance of genetic factors, possibly linked to the epileptic condition with regard to minor anomalies, are observations from a study dating from before the introduction of currently used antiepileptic drugs, in which epicanthus, low set ears and dermal arches were more frequently observed in epileptic patients themselves (53). Further elucidation of the relation between epilepsy and minor anomalies will be obtained as soon as the genes encoding for (predisposition to) epilepsy have been identified, and their segregation can be studied along with that of minor anomalies within families with epilepsy.

1.1.7 Functional impairment; somatic and psychomotor development

Besides effects on organogenesis, embryotoxic effects might also be expressed in pre- and postnatal growth as well as in psychomotor development (for extensive reviews see 8 and 11).

Somatic development

Weight, height and head circumferences at birth of children exposed to antiepileptic drugs have been compared with controls or the general population. Although most studies found no significant differences in gestational age and birth measurements between infants of treated epileptic women and controls, some reported a slightly lower birth weight for exposed infants (21, 29, 34, 35, 43).

Several studies reported significantly reduced head circumferences or an increase frequency of microcephaly in infants born to treated epileptic mothers compared with controls (29, 43, 129-133). The lowest value of the mean head circumference at birth was observed in children exposed to carbamazepine and phenobarbital monotherapy, whereas for valproate no reduction compared with controls was found (43, 129, 130). Also exposure to primidone alone, has been associated with an high frequency of microcephaly (131). The final analysis of the results of a Finnish study group (132) showed however, that with adjustment for parental head circumference the observed difference was not significant any more, even though the barbiturate exposed infants continued to have the lowest mean value. Thus, although a mild drug effect from barbiturate and carbamazepine cannot be excluded, genetic causes may contribute to the small head circumferences observed in some exposed infants. Other factors that appear to contribute to the decrease in head circumference are cigarette smoking, low socioeconomic class, major seizures during pregnancy, and polytherapy (133).

A decrease in biparietal diameter in exposed fetuses was not detected by ultrasound examination at 16 weeks, but became visible from 20 weeks onwards (134). Most of the birth head circumferences values remain within the normal limits. Granström and Hiilesmaa (132) reported that the reduced mean head circumference still existed at examinations at 18 and 30 months, whereas Ogawa and coworkers reported that in most infants it was normalized after 6 months, but in some had not normalized at 3 years of age (135).

Mental and psychomotor development

In many early studies on antiepileptic drug exposure during pregnancy, infants with mental retardation have been described (13, 38, 27, 28, 41, 123, 128). Many of these children also showed other malformations, minor anomalies, or growth retardation. Several of these reports have suggest an association with the treatment.

Results from these studies must be accepted with care. The important field of neurobehavioral effects of antiepileptic drug exposure *in utero* and postnatal development is still in its infancy. The nature and extent of retardation, and the possibility of rehabilitation, with follow-up until puberty, are all subject for further study. Possible adverse effects on functional development is not restricted to first trimester exposure, but extends over the whole duration of pregnancy as well as post natal life, in which even exposure through breast feeding may play a role. Disease related or antiepileptic drug induced changes in maternal mental state might influence the mother-child relationship. Besides drug exposure, all possible factors, which may influence intellectual performance should be taken into account, including genetic background, the educational level and socioeconomic conditions of the family, the disease state and possible sedation of the mother.

Fetal behaviour has been compared between fetuses exposed to antiepileptic drugs and control fetuses, by ultrasound examination, fetal electrocardiogram and fetal movements perceived by the mother. Both groups showed similar patterns, except that exposed fetuses showed a slightly lower fetal hart rate, and fewer fetal eye movements, and the quality of fetal motility was also considered slightly impaired (136). Distinguishing between transient effects of the antiepileptic drug exposure at the time of examination and the possibility of persistent effects on central nervous system development remains difficult. Moreover, the predictive value of fetal behaviour on postnatal psychomotor development remains ambiguous (136).

Recent prospective studies (137-140), in which developmental testing was performed in infants born to treated epileptic mothers showed a slightly increased risk for mental subnormality. In the Finnish prospective study, 121 children of women with epilepsy, mainly exposed to phenytoin *in utero*, and children of a control group of the same size, were examined at the age of 5.5 years. Mental deficiency was observed in 1.4% of the study group, which was not significantly different from the general population. Nevertheless, the mean intelligence quotient was significantly lower in

children of the study group compared with that in control children, but no association was found with fetal exposure or short maternal convulsions. A high number of minor anomalies was associated with a lower mean intelligence quotient in children of both groups. Nevertheless, minor anomalies which could be related to the fetal phenytoin syndrome, were not indicative of low intelligence in affected infants (137). Significantly more children of the study group than controls had some type of cognitive dysfunction which was associated with maternal partial seizures, seizures during pregnancy and paternal education, but not with antiepileptic drug exposure *in utero* (138).
In a well controlled prospective study, Yerby *et al.* (139) observed a significant delay in language skills with a vocabulary of only 50% of controls in exposed infants.

A Japanese study group tested 45 infants of variable age, all exposed *in utero* to polytherapy, and observed more psychomotor retardation, and less well developed social and linguistic abilities, which were more pronounced in the children born to women with partial seizures. In this study an association was found with number and doses of the antiepileptic drugs used, but also with maternal education level and mother-child relationship. The effects were more pronounced in the older (24 months) than in the younger children (140). This seems contrary to most of the currently available literature, which suggests that adverse effects on the child's development of antiepileptic drugs might be apparent especially in early life, but in the long run the rearing environment appears to be more important (8, 141).

1.1.8 Methodological considerations with respect to epidemiologic studies on birth defects after antiepileptic drug exposure.

When data from the literature are combined several possible bias might occur.
Since negative studies are less likely to be submitted or accepted for publication, publication bias can never be excluded. However, since most studies concerning antiepileptic drug use during pregnancy in relation to outcome evaluate a variety of malformations observed in association with a variety of antiepileptic drugs, the impact of publication bias is most probably less than for studies which try to answer a narrower, more specific question.

Bias in the form of selective reporting of the exposure and outcome is a more likely phenomenon, especially when specific malformations are observed or specific combination therapies are used more frequently. An initial report about a possible association probably stimulates other clinicians to report cases with the same anomaly after the same exposure. However, if both events would be a chance finding, similar results would be extremely unlikely to be observed in other studies.

Differences due to study design, base population, outcome ascertainment, length of follow-up, etc. complicate direct comparisons between different studies. Therefore, summary measures calculated by simply pooling numerators and denominators from individual studies should be discouraged (142). However, when we can verify that the groups under comparison within an individual study are treated equally, comparison of

intra-study relative risks might be less affected and study stratified summary relative risk point estimates and associated 95% CI using Mantel-Haenszel procedure can be calculated. With this procedure individual study risks are weighed according to their precision. Even if the intra-study relative risk is zero or undefined (when an specific treatment or malformation is not reported in a study), it still contributes to this relative risk summary estimate. This approach have been used for example by Rosa (68) to compare the risk of neural tube defects associated with valproate and carbamazepine, based on the pooled available data.

Bias due to the design of the study might also be expected. Selection bias between cases and controls might effect the results, especially in case-control studies. Overall malformation rates appear to be slightly higher in prospective studies compared with retrospective cohort studies. This might be explained by inclusion of malformations which are not readily detected at birth and thereby missed in the retrospective survey.

Bias due to source differences in study population might occur as well. In studies based on epilepsy clinics, more severe or treatment-resistant epilepsy cases are expected which could lead to the use of higher doses and more combination-therapy. On the other hand, epilepsy-clinic based studies are depending on birth defects reported to them and follow-up might be incomplete. Selective ascertainment might also influence data from birth defect registries and in this type of studies exposure data are more often poorly described.

Type of epilepsy may confound a possible relationship between a specific drug and a specific malformation or overall malformation rate, if specific types of epilepsy would be most often controlled with a specific drug and this specific type of epilepsy would result in higher rates of specific major malformations. Only very few reports in the currently available literature provided direct information on the type of epilepsy. On the other hand, a detailed subdivision according to the complexity of classification of epileptic seizures and epilepsies, would result in many subgroups with limited numbers, which makes such analysis impossible. Probably only crude subtypes of epilepsy can be used to maintain statistical power. Prescription of antiepileptic drugs for specific types of epilepsy varies strongly across time and place, probably due to different registration procedures, market pressures and individual experience (44), but there is no reason to expect marked differences in prevalence of different type of epilepsy between these populations. Should this confounding exist, it should be greatly diluted in the pooled data of studies from different locations conducted over the last three decades, and it would therefore be unlikely to bias the results of the overall increased malformation risk observed with antiepileptic drug exposure. On the other hand, it might be an additional argument why we are not able to distinguish between the different antiepileptic drugs used.

An other often mentioned potential confounder is the severity of the epilepsy, which is difficult to quantify. Higher anomaly rates might be expected in epilepsy-clinic based studies, based on the assumption that individuals with more severe and/or less well to control epilepsy would be more likely treated in specialized clinics, with higher doses and with combinations of antiepileptic drugs. Against this expectation most epilepsy clinic based studies reported lower rates of anomalies than either hospital or population based

studies which, as mentioned before, might also reflect shorter or less detailed follow-up.

Overall malformation rates and type of malformations observed and reported are greatly influenced by the definitions used for major anomalies, the inclusion of minor anomalies, the method of ascertainment used, and length of follow-up period. Even the standardized coding system commonly used for congenital anomalies (the International Classification of Diseases) contains no provision for differentiation of the severity of most birth defects. If treatment is available, the fact that treatment was given might be used to indicate the severity of the defect, *e.g.* for hipdysplasia and inguinal hernia.

Methodological problems are even more pronounced when minor anomalies or developmental delay are studied. The distinction between a pathological deviation from the norm and a deviation within the normal range of individual variation within the population is extremely difficult to make. Identifying minor anomalies, without a standardized checklist, criteria for the anomalies, corrections for parental measurements, an appropriately defined group of controls, and an observer which is unknown with the antiepileptic drug exposure, is extremely sensitive to bias.

Moreover, evaluation of psychomotor or mental development is even more complicated, since any functional abnormality is likely to be of a complex background, involving both neurobiological factors as well as environmental interactions. Developmental testing is further complicated by selection of the appropriate tests, performed on the most suitable age, their reliability, utility and sensitivity, and with sufficient subclasses to identify specific problem areas. Thereby, attempts to control some of these unknown factors by properly designed animal experiments on functional development introduce probably even more uncertainties, since the relationship of developmental events before and after birth differ markedly with species (143) and translation of a particular test result to the human situation is far from clear.

One of the major problems in studying birth defects in relation to drug exposure is that individual studies hardly reach sufficient numbers for statistical power. One way to overcome this problem is meta-analysis which makes use of the extensive amount of available data collected on the subject up to date and which gives full attention to potential bias. However, to do so more clinical data concerning epileptologic and genetic background are required. The ideal method however, would be large collaboration of groups conducting population-based prospective studies, over large periods of time, using the same study design without methodological shortcomings, with strict definitions of congenital malformations and follow-up period, execution and presentation.

Some basic study design requirements should be fulfilled. Those requirements are: a medical diagnosis and description of epilepsy and family background, precise description of antiepileptic drug use including dosage and preferably plasma concentrations, consistent follow-up among the different groups under comparison, precise definitions of major and minor birth defects, ascertainment of birth defects that appeared to be independent of antiepileptic drug exposure (or at least equal among comparison groups), and loss of follow-up which appears to be independent of epilepsy status and anticonvulsant exposure.

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POSSIBLE MECHANISMS INVOLVED IN THE TERATOGENESIS OF ANTIEPILEPTIC DRUGS

1.2.1 Introduction

The vast majority of experimental studies devoted to explain the mechanism by which antiepileptic drugs interfere with normal development have been focused on the ability of phenytoin to induce orofacial clefts and valproic acid to induce neural tube defects, mainly since these defects can be replicated in a reproducible manner and with relatively high frequencies in animal models.

1.2.2 Phenytoin-induced cleft palate

Cleft palate can be experimentally induced in mice, rats and cat by phenytoin administered during the critical period (for a listing of all animal studies conducted on this topic, see reference 1). Susceptibility to phenytoin-induced cleft palate varies between different inbred mice strains. Of the tested strains, A/J mice appear to be the most sensitive (2, 3), only requiring subcutaneous doses of 12.5 $mg.kg^{-1}.d^{-1}$ (4) during day 9 to 15 of gestation. Different inbred mice strains show distinct differences in response frequency with respect to different types of malformations, leading to differences in the observed pattern of malformations (5). These data support genetic variation in the susceptibility to specific phenytoin-induced malformations. Studies have tried to correlate these differences in susceptibility to phenytoin-induced teratogenesis with differences in metabolism (6), or differences in glucocorticoid receptor levels (7, 8).

Reactive intermediates; phenytoin-derived arene oxides and/or free radicals

One of the proposed mechanisms for the teratogenic action of phenytoin is the generation of reactive intermediates in the metabolism of the drug.

The existence of the dihydrodiol metabolite (5-(3,4-dihydroxy-1,5-cyclohexadien-1-yl-5-phenylhydantoin) of phenytoin, is indirect evidence for the formation of an epoxide

intermediate in the metabolism of phenytoin (9). In general, arene oxides are electrophilic reactive species, and inherent to their chemical reactivity they may covalently bind to nucleophilic sites in cellular macromolecules. This could interfere with normal cell function or if this irreversible binding occurs within the embryo at critical periods of development with normal morphogenesis. In view of the known cytotoxic, mutagenic and teratogenic properties of some epoxides (10), this hypothesis has been given great attention.

Radiolabelled phenytoin can cross the placenta and become covalently bound to fetal tissues (11), detected as the parent drug or several metabolites (12). Formation of these potentially reactive metabolites could be located in maternal tissue, most likely the liver, placental tissue or in the fetus, since the human embryo and fetus are capable of some drug metabolism (13). If the reactive species should be formed within the maternal liver, it should be stable enough to be transported to the fetal organism. In case of the arene oxide of phenytoin it might be tautomerized to a more stable oxepin, which can then cross the placenta and isomerize back to a reactive intermediate (14).

More evidence providing support for the hypothesis of oxidative metabolites being critical in mediating the teratogenesis of phenytoin, comes from experiments with enzyme modulators. Inasmuch as different mice stains show different sensitivity to phenytoin-induced teratogenicity and respond differently to several inducers or inhibitors, data seem conflicting, but most of the experiments conducted with the CD-1 mice give consistent results.

Inhibition of epoxide hydrolase (E.C. 3.3.2.3) - one of the metabolic pathways for arene oxide intermediates leading to dihydrodiol formation - by 1,2-epoxy-3,3,3-trichloropropane (TCPO), doubled the frequency of cleft palates in CD-1 mice (11, 15), but had no effect in C57 mice (16), a strain which is less sensitive to phenytoin-induced cleft palate (5). Induction of cytochrome P-450 by pretreatment with phenobarbital increased phenytoin teratogenicity in CD-1 mice, whereas inhibition by SKF 525A pretreatment resulted in significantly decreased cleft rates in CD-1 mice (15), supporting the "arene oxide hypothesis". Paradoxically, however, in Swiss-Webster mice (SWR) pretreatment with these same cytochrome P-450 modulators gave opposite results, and the authors suggested, at that time, that the parent drug itself was the ultimate teratogen (17).

Conjugation with glutathione, eventually leading to mercapturic acid metabolites, is another possibility when epoxide intermediates are present, although subcellular localization might favour a reaction catalyzed by epoxide hydrolase (18). Thus far, no such metabolites of phenytoin have been identified. Besides direct detoxification of reactive metabolites by conjugation, glutathione may also protect against oxidant stress and/or lipid peroxidation, which might be initiated by free radical intermediates.

Evidence to support glutathione involvement comes from experiments demonstrating an increase in covalent binding of phenytoin and an increase in phenytoin-induced cleft palates in CD-1 mice pretreated with compounds known to deplete glutathione stores, like diethyl maleate (19), and acetaminophen (20). Furthermore, a slight depletion of hepatic glutathione synthetase in pregnant mice is observed (20). Co-administration of N-acetylcysteine, a glutathione precursor which might compete with

other thiol-groups for binding with reactive intermediates, before or after phenytoin treatment, showed variable effects on phenytoin-induced cleft-palate rates and was embryotoxic at high dose levels (21).

Evidence for a prostaglandin H synthethase-catalyzed free-radical intermediate of phenytoin, centred in the hydantoin nucleus, has also been described (22). Free radical intermediates may bind covalently to essential proteins or nucleic acids, cause oxidant stress or may initiate lipid peroxidation reactions. If this occurs during a critical period normal development might be disturbed (22).

Prostaglandine H synthetase is a bifunctional microsomal protein, containing both a fatty acid *cyclooxygenase*, which oxygenates arachidonic acid to prostaglandine G2 (PGG2) and a *hydroperoxidase*, which subsequently reduces this PGG2 to prostaglandin H2 (PGH2) (23). Several other compounds can substitute the endogenous cofactors, involved in the latter reaction, being cooxidized to reactive electrophilic intermediates. Whereas cytochrome P-450 activity in the embryo is rather low, prostaglandin H synthetase activity is high in embryonic tissues (24).

In *in vitro* experiments, in the presence of α-phenyl-N-t-butylnitrone (PBN), a free radical spin trapping agent, the radical could be stabilized and identified with electron spin resonance spectrometry (25).

The incidence of cleft palate (*in vivo*) and covalent binding of phenytoin (*in vitro*) could be reduced with several prostaglandin H synthetase inhibitors (like indomethacin and acetylsalicylic acid), or free radical scavengers, like PBN, or antioxidizing agents, like caffeine as well as with glutathione (26). Concomitant treatment with 12-O-tetradecanoyl-phorbol-13-acetate (TPA), an activator of phospholipase A_2 (25) enhanced phenytoin-induced teratogenicity. This phospholipase releases arachidonic acid from cell membranes and thereby enhances prostaglandine H synthetase activity, which in turn could enhance phenytoin bioactivation through this route.

Since the free radical appears to occur within the hydantoin nucleus, this "free radical hypothesis" would allow a unifying hypothesis to explain the teratogenicity of all hydantoins and structurally related antiepileptic drugs, such as trimethadione and dimethadione who do not posses phenyl-substituents and therefore cannot form arene oxide intermediates (25).

Phenytoin-induced alterations in prostaglandins synthesis

Another line of research has found evidence for interactions with the glucocorticoid receptor, leading to alterations in prostaglandins synthesis.

It has been proposed that phenytoin may produce cleft palate by a mechanism similar to glucocorticoids by interference with glucocorticoid-receptor mediated prostaglandins levels (7, 8). Both substances delay horizontalization of embryonic palatal shelves (27, 28) and inhibit programmed cell death and lysosomal activity of the medial epithelium in sensitive embryonic palates in organ culture (29).

Competitive binding of phenytoin to the glucocorticoid receptor is demonstrated

(30). This binding mediates, in analog with glucocorticoids, the production of a phospholipase A_2 inhibitory protein, leading to a decline in release of arachidonic acid from cell membranes. Subsequently lower arachidonic acid levels are available for prostaglandins and thromboxane biosynthesis (31), which might interfere with normal palate development (32). Coadministration of arachidonic acid and glucocorticoids reduces the teratogenic potency of the steroids in mice *in vivo* (32) and phenytoin-induced defects in facial arches, head fold and neural tube fusion in 8.5 day-old mice embryos cultured for 48 hours *in vitro* (33).

In mice, susceptibility to glucocorticoid-induced cleft palate is influences by 2 genes within the H-2 histocompatibility locus on chromosome 17. These same genes appear to influence glucocorticoid receptor levels in embryonic palate (34). Differences in susceptibility to phenytoin-induced cleft palate correlated also with H-2 histocompatibility-linked genes, with receptor expression being higher in the susceptible H-2a strain than the "resistant" H-2b congenic strain (8). Since levels of glucocorticoid receptors in peripheral lymphocytes were elevated in the susceptible mice, they tested human lymphocytes of exposed infants and indeed found higher receptor levels in the infants affected with the fetal hydantoin syndrome than in unaffected children with similar exposure in control families. The glucocorticoid-receptor levels of these affected infants were also elevated compared with levels of parents of both sexes in these control families and fathers of children with signs of the fetal hydantoin syndrome. The mothers of affected infants, however, showed large variation in receptor levels, with a mean value intermediate between affected infants and fathers of affected offspring (35).

Another group studying genetic differences in susceptibility to phenytoin-induced orofacial clefts found, albeit a significant associated with the major histocompatibility locus H-2, no correlation with any glucocorticoid receptor parameter or levels in cAMP in palates of day 15 mice fetuses (36). Nevertheless, studying the human equivalent of mice H-2, the major histocompatibility complex (MHC or HLA), in relation to phenytoin-induced teratogenicity might be of interest.

Interference with folate metabolism

Folate metabolites are important for: the transfer of one-carbon units of various reduction states (*i.e.* formyl, methyl); the biosynthesis of purines and pyrimidines; the metabolism of several amino acids such as glycine, serine, homocysteine, methionine and histidine; and in methylation of DNA. Interference with these essential processes could impede normal development especially during the rapidly proliferating and differentiating early embryo (37).

Almost as old as the discussion whether antiepileptic drugs are teratogenic, is the speculation that they might do so by interference with folate metabolism (38). From the early sixties onwards, abnormal serum folate levels (sometimes even with megaloblastic anaemia) in association with antiepileptic drug use have been described, which has resulted in an extensive literature on folic acid, antiepileptic drugs and epilepsy itself (for

review see 39). Malnutrition and pregnancy can act as predisposing factors for this folate deficiency.

Folate deficiency is especially seen with phenytoin, but also with phenobarbital and primidone, and more often when combinations of these drugs are used (39, 40, 41). For carbamazepine and valproate there is no strong evidence for abnormally low serum folate levels, but several studies report some form of interference with folate metabolism (42-44). The mechanism by which phenytoin causes folate deficiency is still not elucidated. Several suggestions have been made of which malabsorption from the gut is the most likely explanation. Others possibilities are: a general induction of liver enzymes, induction of enzymes involved in folic acid metabolism, competition with folate coenzymes or an increased requirement of folic acid due to hydroxylation of phenytoin itself (41). For carbamazepine and valproate inhibition of intestinal folate absorption is also suggested (42-44), although this does not result in a general reduction in serum folate levels.

Other studies have implicated folate deficiency in the general population as a possible risk factor for congenital malformations in general (45, 46) or neural tube defects, in particular (47). Similarities have been reported between the pattern of phenytoin-induced malformations, *i.e.* cleft palate, skeletal defects and growth retardation, and abnormalities seen after exposure to aminopterin or other folic acid antagonists, both in experimental animals (48, 49) and human (50, 51). Experimentally induced mild folate deficiency by feeding the animals a folic acid deficient diet resulted especially in growth retardation and fetal wastage (52).

The modifying effect of folic or folinic acid coadministration on phenytoin-induced cleft palate has given variable results, both between and within studies, which in the latter seems to be partly dose-related. Martz and Fraser found a protective effect on cleft palate rates in mice with folinic acid, but not with folic acid coadministration (53). Also Kernis and coworkers did not observe a reduction in cleft palate incidence with folic acid (54). In three studies a dose-related variable response to folinic acid coadministration was observed. Whereas at low doses some (55, 56) reduction in phenytoin-induced cleft palate frequencies were observed, at higher doses folinic acid supplementation actually potentiated the teratogenic effects of phenytoin (55, 56, 57).

Billings and Hansen showed that teratogenic doses of phenytoin were associated with alterations in relative concentrations between different folate metabolites. In the liver of treated dams, they observed increased concentrations of tetrahydrofolate in combination with decreased concentrations of 5-methyltetrahydrofolate, and were able to demonstrate decreased activity of the relevant converting enzyme, 5,10-methylenetetrahydrofolate reductase. Since, 5-methyltetrahydrofolate is the folate form that circulates in plasma, this would also explain the observed reduced total folate levels in plasma (58, 59).

Three prospective study groups have included evaluation of folate status in pregnancies with antiepileptic drug use in relation to pregnancy outcome. The study groups in Montreal (60) (four early spontaneous abortions and five with developmental anomalies of 49 evaluated pregnancies) and Japan (61) (seven malformed of 51 evaluated pregnancies) found significantly lower serum and red cell folate levels periconceptionally

(60) or in the first and second trimester (61) in mothers with malformed offspring. In a larger study in Finland (62) (10 structural defects, 10 with some sign of the fetal hydantoin syndrome and 5 perinatal deaths among 133 evaluated pregnancies) no such association was found, albeit the observed negative correlation between serum folate levels and serum concentrations of phenytoin and phenobarbital. No oral clefts or neural tube defects were observed. In the Finnish study 91% of the women took folic acid supplementation (mean dose 500 $\mu g.d^{-1}$ (range 100 - 1000 $\mu g.d^{-1}$) from the 6th week of pregnancy onwards. Serum folate levels were determined from 8th to 16th week of pregnancy. Thus, this supplementation might have obscured a possibly existing, mild folate deficiency in an earlier period of organogenesis.

In conclusion, much effort has been directed to the possible role of folate deficiency in birth defects in general, as well as in association with antiepileptic drugs use. However, no conclusive evidence has been obtained up to date, most likely because of the complexity of folate metabolism and the lack of understanding of the precise role of different folate forms during development, and the precise interference of antiepileptic drugs with folate metabolism. The occurrence of neural tube defects has also been associated with folate deficiency and therefore this topic will arise again, in relation to valproate-induced neural tube defects.

1.2.3 Valproic acid-induced neural tube defects

Valproic acid has the potential to induce neural tube defects in the mouse, hamster, rhesus monkey and human. Whereas in humans an increased risk for posterior neural tube defects is observed, in rodents it is especially the anterior neural tube that fails to close (see for review 63). Only recently, posterior defects have been experimentally induced in animal models in the rhesus monkey (64) and the mice (65). The latter study showed only low frequencies of spina bifida aperta at doses 10 fold higher than human therapeutic doses (based on body weight), but a much higher incidence of the occult form (65). The daily doses needed to elicit these teratogenic effects in experimental animals were about 10 folds the usual therapeutic dose in humans based on body weight, but equivalent based on body surface (63).

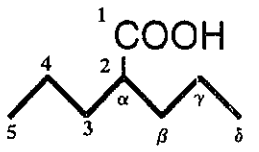
After treatment of pregnant mice on day 8 and 9 of gestation with ^{14}C-labelled valproic acid it could be demonstrated with autoradiography and computerized densitometry that the embryonic neuroepithelium was the target organ, where irrespective of dose the highest concentrations were found. However, a disproportionately increased accumulation at higher dose levels was observed, and it was concluded that with these high doses of valproate saturation of the capacity of metabolism, excretion, and protein binding in the maternal organism resulted in more of the drug to accumulate in this target tissue, the embryonic neuroepithelium (66).

Genetically determined differences in susceptibility to valproic-acid induced neural tube defects have also been demonstrated, with an observed hierarchy of the three tested inbred mice strains, of SWV > LM/Bc > DBA (67).

The parent drug is the ultimate teratogen

The induction of exencephaly in mice by various analogues and metabolites of valproic acid has been shown to follow strict structural requirements (fig 1.2.1) (63). To be teratogenic, the compound must possess a free carboxyl group connected to the α-carbon atom (C-2), an α-hydrogen atom, and branching of the carbon chains at that α-carbon atom. The alkyl substituents on C-2 should preferably be 3 carbon atoms each, and without double bounds between C-2 and C-3, or C-3 and C-4 (68). Introduction of a double or triple bound between C-4 and C-5 enhances the teratogenic activity (69, 70). By introduction of this double bound, the α-carbon become asymmetric (*i.e.* binding to four different substituents) resulting in enantiomer formation. The difference between both enantiomers was most pronounced for 4-yn-valproic acid (*i.e.* a triple bound), with the S-enantiomer being about eight times as potent as the R-enantiomer (70). This enantioselective teratogenicity appears to be related to differences in intrinsic activity (63).

Fig. 1.2.1 Valproic acid



Polar substituents appear to decrease the teratogenicity as the hydroxylated metabolites were found to be inactive (68, 71). More evidence to support the hypothesis that the drug itself is the ultimate teratogen comes from *in vitro* studies, in which valproic acid is active in the cultured rat or mouse embryo, where little metabolic activity can be expected (72, 73). *In vivo,* concentrations of metabolites in the embryo are extremely low and pretreatment with phenobarbital, which induced valproic acid metabolism reduces the teratogenicity of valproic acid (74). Comparison of various routes of administration (subcutaneous, oral, intraperitoneal, implanted minipumps) showed that the teratogenicity corresponds to the plasma concentrations of the parent drug (74, 75).

Morphologic investigations into the pathogenesis

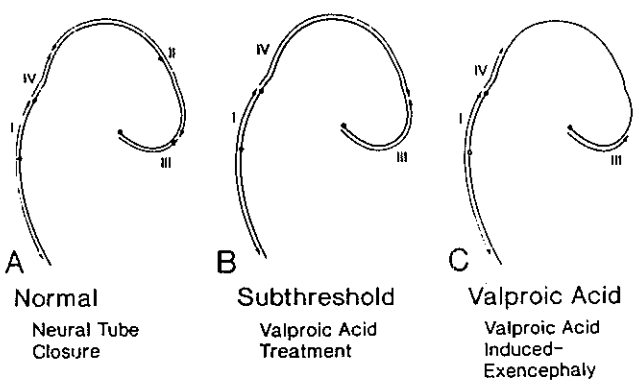
In mouse embryogenesis, neural tube closure is a four initiation points process (75, 76). Treatment of the inbred mice strain SWV, which is sensitive to valproic acid-induced neural tube defects, with a single 600 $mg.kg^{-1}$ dose of valproic acid on gestational day 8:12, showed an alteration in this closure process in the valproic acid treated embryos. With valproate closure II fails to complete, resulting in non-closure of the neural folds in the region of the prosencephalic-mesencephalic border. When closure III, which arises from the stomodeum and spreads caudally, is completed, the embryos have a closed prosencephalon in the region of the anterior neuropore, yet the mesencephalon remains open, resulting in exencephaly. Subthreshold doses of valproic acid lead to less developmental delay, and compensation can take place by extending the caudal limits of

closure III and the rostral limits of closure IV (fig. 1.2.2)(67).

When ^{14}C-labelled valproate was administered to pregnant mice on day 8 and 9 of gestation, a strong labelling of the embryonic neuroepithelium was found (66). Histologically, the normal architecture of the neuroepithelium is disturbed, with a loss of integrity at both the basal and apical surfaces. After treatment with 340 $mg.kg^{-1}$ valproate, i.p. on day 8 to CD-1 mice, evaluation of the neuroepithelium on day 10 revealed marked disorganization, with a loss of radial arrangement of the epithelial cells
and a loss of intracellular adhesion in the juxtaluminal region. Mitotic activity was sometimes not confined to the ependymal layer, any more, but was scattered throughout the neuroepithelium (ependymal, mantle, marginal). Furthermore, a marked decrease in vascularity in the neuroepithelium in the hindbrain region of treated embryos was evident and several of the embryos showed a thinning of the forebrain neuroepithelium. The lumen was irregular in shape, and contained blood cells from blood vessels with apparently ruptured endothelial lining. The underlying basal lamina also showed irregularities and gaps (78). Since, the basal lamina has been shown to be important in morphogenetic shaping of the neural tube and in established polarity in the neuroepithelium (79), the alterations observed in the basal lamina could contribute to the cellular disorientation in the neuroepithelium and dysmorphogenesis of the neural tube. The authors suggested that the loss of cellular adhesion in the juxtaluminal region could reflect effects of the drug on intracellular junctional complexes and contractile microfilaments (78).

Valproate has been shown to inhibit cell growth and induce differentiation *in vitro*. Valproate impaired the mitotic rate in neuroblastoma (Neuro-2A) and glioma (C6) tumour cell lines with an IC_{50} of approximately 0.5 and 1 mM, respectively, without apparent cytotoxicity (80, 81). Continued exposure (1 to 3 weeks) to 1 mM valproate induced differentiation in both cell lines and a 2.5 fold increase in cell-substratum adhesion in C6

Fig. 1.2.2 Altered sequence of neural tube closure in the mouse following valproic acid treatment (From Finnell RH: Genetic differences in susceptibility to anticonvulsant drug-induced developmental defects, Pharmacol Toxicol 1991;69:223-7, reproduced with permission)



glioma cell lines. When mouse neuroblastoma X rat glioma hybrid cell cultures (NG108-15) were exposed for 4 days to phenobarbital (20-120 $\mu g.ml^{-1}$), phenytoin (2-50 $\mu g.ml^{-1}$), carbamazepine (2.4-24 $\mu g.ml^{-1}$), or valproic acid (10-200 $\mu g.ml^{-1}$), cell growth was reduced with valproic acid and phenytoin. Valproic acid acted as a differentiating agent, as measured by an increase in activity of choline acetyltransferase, ß-galactosidase, and muscarinic cholinergic receptor binding (82). The observation that valproate inhibits cell growth and induces differentiation is comparable with other agents with established teratogenic properties (83).

Several postulated mechanisms; pH, zinc metabolism, and embryonic lipid metabolism

Several different hypotheses on the mechanism of valproic acid-induced neural tube defects have been generated, ranging from very general to more specific ones. It has been suggested that weak acids may alter embryonic pH, which is a critical determinant of a number of fundamental processes, including cell proliferation and differentiation and intercellular communication (84). Since, the pH in the developing early embryo is approximately 0.4 units above that of maternal blood, weak acids, like valproic acid, will accumulate up to 2 fold in the embryo via ion trapping (85). Whether this accumulation will indeed lead to changes in embryonic pH remains to be established. By now, this general postulation for teratogenic potential of acidic compounds seems contrary to the aforementioned strict structural specificity of the teratogenic action of valproic acid and related carboxylic acids.

Since it was pointed out that the incidences of spina bifida and anencephaly were highest in some of the areas of the world where human zinc deficiency exists (86), interference with zinc metabolism has also been suggested as a mechanism by which valproic acid induced neural tube defects. However data are fragmentary. Valproic acid reduces serum zinc concentrations in mice (87), but has no effect on maternal and fetal serum zinc concentrations in rats (88). Zinc supplementation did not prevent the teratogenic action of valproic acid on cultured rat embryos (89).

Interference with embryonic lipid metabolism has also been suggested as a possible mechanism for valproic acid-induced teratogenicity (90).

Interference with folate metabolism

Folate deficiency in the general population has been implicated as an etiologic factor in the occurrence of neural tube defects (47). Based on these findings, folate supplementation, around the time of conception and during the first trimester, has been studied extensively from the early seventies onward. Several large clinical trials have been conducted in relation to the prevention of neural tube defects (91, 92), but controversy remained, mainly due to methodological problems. Only recently this situation seems to have changed, thanks to a large randomized double-blind trial of

women with a recurrence risk for neural tube defects of The Medical Research Council. Folic acid supplementation with 4 $mg.d^{-1}$ from at least one month before up to 2 months after conception, possessed a tremendous protective effect, and reduced the recurrence risk from 3.4% to 0.7% (93).

Yates demonstrated that red cell folate levels of women with a previous infant with a neural tube defect were significantly lower than those found in controls. Women with two previous affected infants had even lower red blood cell folates. Since this association could not be adequately accounted for by the dietary intake, the authors postulated a defect in folate metabolism as the possible cause (94).

A disturbance in homocysteine metabolism, which is interconnected with folate metabolism has been suggested by Steegers-Theunissen and coworkers. After a methionine load they observed high blood homocysteine concentrations in five out of 16 mothers, who had given birth to an infant with a neural tube defect (95). Further investigations showed elevated homocysteine concentrations in only 8 out of 40 mothers. This disturbance could not be explained by cystathionine ß-synthase deficiency and this led the authors to suggest a remethylation defect (personal communication).

Although folate deficiency is predominantly seen with phenytoin treatment, valproate and carbamazepine, which are more specifically associated with neural tube defects, might interfere with intestinal folate absorption leading to slightly reduced folate concentrations (96).

In mice, folinic acid (5-formyl-tetrahydrofolic acid, 5-CHO-THF) coadministration prior to and immediately following a teratogenic dose of valproic acid significantly reduced the incidence of exencephaly to 30-50% of initial rates in most cases (97). However, protection was dependent on the time of day, with no protection in the late morning hours, possibly related to the large diurnal fluctuations of folate metabolites concentrations in the embryo (98, 99). It appears that valproic acid does not alter the total folate concentrations in either the serum of the dam or in gestational tissue, but alters relative concentrations of selected formylated tetrahydrofolates. A reduction was observed in the concentrations of especially folinic acid, but also in 10-formyl-tetrahydrofolate and in 5-methyltetrahydrofolate, while tetrahydrofolate was increased (98, 99). This low folinic acid in combination with high tetrahydrofolate could be the result of a valproic acid-induced inhibition of the respective interconversing enzyme, glutamate formyltransferase, since valproic acid reduced the activity of this enzyme by two-third of its initial value *in vitro* (99). However, *in vitro* whole rat embryo culture experiments could not demonstrate a protective effect of folinic acid coadministration on the valproate-induced teratogenicity (100). It would be interesting to see whether the observed structure-activity relationship also holds true for the inhibition of glutamate formyltransferase.

1.2.4 Reactive intermediates; Carbamazepine- or phenobarbital-derived arene oxides

Animal data on the teratogenicity of phenobarbital and carbamazepine are surprisingly limited. On the analogy of phenytoin teratogenesis, binding of epoxides to fetal macromolecules has also been postulated as a possible mechanism for phenobarbital and carbamazepine induced teratogenesis (101, 102). In the metabolism of both drugs arene oxides could arise (103, 104).

Genetic susceptibility to carbamazepine with regard to this hypothesis, has been tested more specifically in a study with two different inbred strains of mice with different epoxide hydrolase activities (SWR and LM/Bc). No related differences could be demonstrated for the observed teratogenic effects (105). With chronic dietary treatment with doses up to 2000 $mg.kg^{-1}.d^{-1}$, from 2 weeks prior to and throughout gestation, malformations of the central nervous system and urogenital tract could be induced, but only in fairly low frequencies in both strains. This might have limited the power to observe differences in this experiment. Limited response frequencies is a general problem with respect to experimental data on carbamazepine teratogenicity. Malformation frequencies remain low and malformation-specific teratogenicity is hard to demonstrate, which make mechanistic studies hard to conduct and moreover, have probably confined experimental animal data on the teratogenicity of carbamazepine altogether. With treatment during the period of organogenesis none or only small increases in the rate of cleft palate, dilated cerebral ventricles, and growth retardation were observed, both in mice and rats (1).

Only four studies have reported on the teratogenicity of carbamazepine in mice. In three of these studies CD-1 mice were used. Paulson found a nonsignificant increase in cleft palate at dietary doses up to 1664 $mg.kg^{-1}.d^{-1}$ given on day 7 to 12. A dose, which is more than 1000 times the human equivalent dose, based on body weight, or about 12 times, based on body surface (106). Sullivan and McElhatton observed only a small increase in the incidence rates of cleft palate, enlarged cerebral ventricles and club feet with oral doses up to 240 $mg.kg^{-1}.d^{-1}$ administered on day 5 to 15 of gestation (107).

In those experiments where the teratogenic potential of different antiepileptic drugs were compared, carbamazepine showed the lowest potential both in mice (105-109) and rats (110, 111).

In a comparison of phenobarbital and phenytoin in three different inbred mice strains (SWV, LM/Bc and C57BL/6J) it appeared that phenobarbital induced a higher frequency of structural malformations, like cleft palate, or defects in the urogenital tract or heart, whereas phenytoin induced more signs of incomplete development, related to growth impairment, such as skeletal ossification delays, dilated cerebral ventricles and hydronephrosis (112).

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AMNIOTIC FLUID REGULATION AND THE DISTRIBUTION OF DRUGS

1.3.1 Introduction

Most of the early data published on the origin, circulation and composition of amniotic fluid comes from samples obtained at or near term, after it became clear that amniotic fluid could be used in the diagnosis and treatment of rhesus-antagonism (1, 2). With increasing interest in and development of antenatal diagnosis of hereditary disorders from the late sixties onward, more data became available from earlier periods of gestation. At present, amniocentesis performed at 16 to 20 weeks of gestation, provides amniotic fluid of which the cultured cells allow us to identify numerical and structural chromosomal abnormalities and about a 100 inborns errors of metabolism (3, 4). The supernatant can be used for the analysis of α-fetoprotein concentrations to detect open neural tube defects. Sampling of chorionic villi earlier in gestation (12 weeks) for analysis of chromosomes, DNA and enzyme activities is now partly replacing amniocentesis. However, for the prenatal diagnosis of open neural tube defects amniocentesis remains important.

Since amniotic fluid surrounds the fetus directly, all kinds of physiological processes in the fetus can, in a more or less distinct manner, manifest themselves in its composition. For example, in methylmalonic aciduria, the affected fetus produces an increased amount of metabolites, which are excreted in the amniotic fluid and finally into the maternal urine.

For any toxicological evaluation a precise description of exposure is essential. Both ethical and experimental limitations confine direct assessment of concentrations in fetal plasma or ultimately in the fetal target tissue. Amniotic fluid, as a compartment for fetal drug distribution, might provide useful information, taking into account that the composition of amniotic fluid varies with gestational age and amniotic fluid obtained at 16 to 20 weeks of gestation, may not be representative for the situation at the time of organogenesis, *e.g.* neural tube closure in human is completed in the fourth week after conception, even before placental function is established. For the interpretation of results on the distribution of drugs in the amniotic fluid, a better understanding of the origin, regulation and composition of amniotic fluid is required.

1.3.2 Origin, regulation and composition of amniotic fluid

The amniotic fluid may arise through secretion or ultrafiltration from the mother directly across the membranes, from the placenta or cord or from the fetus, through the skin, the kidneys, or gastrointestinal or respiratory tract. The importance of the contribution of these different routes may vary over different periods of gestation (5-7).

Early in gestation, the amniotic fluid is probably an transudate of maternal plasma. Amniotic fluid is already present in the early embryo when the tissues of the fetus are poorly differentiated. This suggests an origin via the membranes, with the possibility of active secretion through the amnion (5-7). Immunological studies on proteins have demonstrated both fetal and maternal contributions to the amniotic fluid (8).

When the placenta is established, exchanges can take place through the whole fetoplacental unit of the fetus, cord and placental plate (9). From that time onwards up to about mid-pregnancy, the composition of amniotic fluid suggests that it is an extension of the fetal extracellular space (10).

The fetal skin is permeable to water and some solutes up to 18 to 20 weeks of gestation. Thereafter, the skin looses its permeability due to increasing thickening and keratinisation. The morphology of fetal skin during the third and fourth month of pregnancy is compatible with a role in the exchange of water and electrolytes between amniotic fluid and the fetus. Before keratinisation, the cells of the fetal periderm shows striking resemblance to renal tubular cells (11) and are under the influence of vasopressin (12). Moreover, a close relationship was found between amniotic fluid volume and fetal surface area (13). Apart from the skin, the fetal surface of the placenta is also a transfer site and becomes quantitatively more important when the fetus increased in size (14, 15).

A transmembranous pathway, by which ultrafiltrate of maternal plasma crosses the wall of the uterus and the amniochorion directly, has also been suggested. Parmley and Seeds (16) demonstrated that for amnion and chorion leave the diffusion permeability to isotopic water *in vitro* was similar to that of unkeratinized fetal skin. However, since the surface area of the extraplacental membranes is small compared to the surface area of the placenta, the quantitive importance of this route is probably limited (17, 18).

Fetal urine production appears to start around 11 weeks of gestation with an estimated urinary output of approximately 1.2 $ml.d^{-1}$ (19) and this output increased with progression of gestation. The increasing importance of fetal urine to the composition of amniotic fluid is supported by a reduction in osmolality and increasing concentrations of urea, uric acid and creatinine (20, 21). Fetal bladder emptying could be visualized by ultrasonography (22). Several other fetal secretions, *i.e.* from the respiratory and gastrointestinal tract, buccal mucosa and salivary glands, might also contribute to the amniotic fluid, but evidence for those routes is not very substantiated. Although a substantial volume of lung fluid is secreted via the trachea it is not clear whether this enters the amniotic fluid or is directly swallowed by the fetus (23).

With progressed gestation the role of the fetus in controlling amniotic fluid composition and volume becomes more evident. In the period of interest for our study, *i.e.* between 16 and 20 weeks of gestation, the volume of amniotic fluid ranges from 125-

300 ml at 15-16 weeks up to 220-800 ml at 20 weeks (19). The daily increase in fluid volume during this period ranges from 7 to 13 ml. Around that time, the fetus swallows 4 to 11 $ml.d^{-1}$ (19, 24, 25). So, the total volume to be added to the gestational sac ranges from 11 to 24 $ml.d^{-1}$. Fetal urine may provide about 7 to 14 $ml.d^{-1}$, but the remaining 4 to 10 $ml.d^{-1}$ must come from other sources, most likely the fetal surface of the placenta or the umbilical cord. More recent experiments have indeed demonstrated the importance of such an intramembranous-intraplacental pathway, between amniotic fluid and fetal blood, that perfuses the fetal membranes and surface of the placenta (26).

In conclusion, although pathways for exchange of water and some solutes have been demonstrated, the precise mechanism controlling the volume and composition of amniotic fluid is not clearly elucidated. Results obtained with transfer experiments are by no means clear cut. As a few examples, after intravenously injected radiotagged albumin into the mother, it appears initially in a higher concentration in the amniotic fluid than in the fetal blood (27). Amino-acids injected in the amniotic cavity are first detected in maternal plasma instead of fetal plasma (28). The volume of amniotic fluid varies more than its composition. Amniotic fluid is isotonic with maternal plasma early in gestation, but becomes progressively but slightly, hypotonic over the last half of pregnancy, partly due to increasing amounts of hypotonic fetal urine (29-31).

1.3.3 Distribution of drugs in amniotic fluid

Drug transfer from the maternal circulation into the amniotic fluid compartment may occur via the placenta and the fetal circulation. Several lipid membranes have to be crossed. Hydrophylic molecules diffuse through such membranes only if they are uncharged and of a molecular weight below about 100 D. Lipophylic compounds cross these membranes rapidly up to a molecular weight of approximately 600 D, thereafter their passage is delayed and becomes virtually impossible above a molecular weight of approximately 1000 D.

The vast majority of drugs in use today are weak acids or bases. This implies that they are partly ionized in an aqueous solution. In the non-ionized form they can cross lipid membranes at a rate dependent upon their lipid solubility. The proportion of ionized to non-ionized drug present in solution depends on the dissociation constant of the compound (pKa) and the ambient pH. The reported values of pH 7.0 to 7.25 for amniotic fluid in early pregnancy (32) are slightly lower than the values of plasma. Theoretically this might influence the distribution of weak electrolytes in amniotic fluid.

Drugs are variably bound to plasma proteins. The protein content of amniotic fluid of 16-20 weeks of gestation is about 0.3 g per 100 ml (33), which is less than one-tenth of that in maternal plasma. Protein binding of drugs in amniotic fluid might therefore be negligible, and this will reduce the theoretical steady-state concentration.

Renal excretion plays a dominant role in the distribution of drugs into the amniotic fluid, certainly in late pregnancy. Purely water-soluble drugs may be filtered through the glomerulus, and without reabsorption in the renal tubule, be rapidly excreted in the fetal

urine. More lipophylic drugs tend to be reabsorbed in the renal tubule resulting in less excretion through the fetal urine, but they may enter the amniotic fluid more easily by diffusion across the immature fetal skin and fetal mucous membranes or any adjacent membrane. Thereby, some drugs may utilize available active transport systems.

Finally, the amniotic cavity might be viewed as a bag filled with water and thereby represents a very deep compartment, for which direct equilibrium with adjacent compartments is inevitably slow. The circulation of the fluid through the fetus by swallowing and urinary excretion might speed up overall turnover.

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CHAPTER 2

THE PROSPECTIVE COHORT STUDY

AIM AND FEASIBILITY OF THE STUDY

2.1.1 Aim of the study

The objective of this study was to further assess the relative contribution of antiepileptic drug use towards the occurrence of birth defects in a prospective manner by ascertainment early in pregnancy, with full attention to clinical detail and complete follow-up up to three months after birth.

The first objective of this study was to investigate the precise causative relation of valproate exposure and spina bifida, to further delineate the actual risk and to evaluate prenatal diagnosis of valproate-associated neural tube defects. Since the evidence for the association between valproate and spina bifida had mainly been presented in letters to the Lancet or reports of birth defects registries, without full information on clinical details and a discussion of the epidemiological issues including methodological ones, several questions remained open to discussion (1). With the data of our prospective cohort the following questions were addressed: *i*) Is the previously estimated risk of 1-2% for valproate significantly higher than the risk observed with other antiepileptic drug exposures? *ii*) Is the risk of spina bifida after valproate exposure in some way connected with the underlying epilepsy or other identifiable risk factors? *iii*) Is valproate really specifically associated with spina bifida and not with anencephaly? Since in general both defects show a very similar etiology (2), this would be the first demonstration of such specificity in human. In section 2.2, these issues will be discussed based on the results of our prospective cohort and in section 2.3, based on data of 34 cases of neural tube defects, observed in The Netherlands over the period 1972-1991 in association with antiepileptic drug exposure.

Antiepileptic drug use during the first trimester of pregnancy has become an indication for prenatal diagnosis (3). Evaluation of the antenatal diagnostic procedures in this specific patient group would be appropriate. Our experiences over a 5 years period of prenatal diagnosis of neural tube defects after antiepileptic drug use are described in section 2.4. Results of α-fetoprotein determination in amniotic fluid are compared with results of the determination in maternal serum.

For proper clinical management it is essential to know whether the higher frequency of congenital abnormalities observed in the offspring of epileptic women, who

are treated for this disease, is associated with specific modes of therapy or with other factors, such as different types and severities of epilepsy. Since over the years treatment regimens change, *e.g.* polytherapy being substituted by monotherapy and new drugs becoming available, continuous monitoring of outcome of exposed pregnancies remains desirable, especially when full attention is given to other possible risk factors, such as the underlying maternal epilepsy or family history of congenital anomalies (section 2.5).

Most women in this study underwent amniocentesis at approximately 16 weeks of gestation. This allowed us to evaluate in a standardized way in a relatively early phase in pregnancy (although too late for direct extrapolation to exposure during the time of organogenesis), to what extent valproate (section 2.6) and carbamazepine (section 2.7) and some of their metabolites reach the amniotic fluid and how this relates to levels in simultaneously obtained maternal serum. From the pharmacokinetic point of view, the amniotic cavity can be represented as a bag of water (4). It is expected to act as a deep compartment and therefore, equilibrium with adjacent compartments will be slow. Since the amniotic fluid is closely surrounding the fetus, this might provide a measurement of fetal exposure, which is less sensitive to fluctuations in time. Furthermore, we were interested whether metabolite concentrations in amniotic fluid could be indicative for a predisposition to drug-induced birth defects based on metabolic differences. An approach analogous to the direct measurement of characteristic metabolites in amniotic fluid supernatant in the prenatal diagnosis of certain amino- and organic acidurias (5).

Since hyponatraemia in conjunction with carbamazepine use, has been associated with a delay in caudal neural tube closure (6) in whole rat-embryo cultures *in vitro*, we have included electrolyte levels in our evaluation (section 2.8).

2.1.2 Evaluation of the feasibility of the study

Women using antiepileptic drugs during their pregnancy are offered structural examination by ultrasonography around 20 weeks of gestation. With this method some of the defects which occur more frequently in the offspring of treated epileptic women, *i.e.* heart defects and orofacial clefts, can be visualized (7). From 1984 onward, after the association of neural tube defects and valproate exposure became clear (and suspected for carbamazepine exposure), amniocentesis for α-fetoprotein determination in amniotic fluid was offered as well (3).

In november 1985, a prospective study was initiated through the outpatient clinic of prenatal diagnosis to which pregnant women with epilepsy and receiving antiepileptic medication were referred. The Centre for Clinical Genetics for the region South-West Netherlands covers approximately 40,000 births per year (8). With a frequency of maternal epilepsy at confinement of 0.3%-0.4% (9), and a study period of 4 to 5 years we estimated that with a theoretical participation rate of 100%, the maximal number of patients that could be expected would be 570. Yet, a participation rate of about 50%, which has been calculated for women over 36 years of age, who use the offered possibility for prenatal diagnosis for chromosomal abnormalities (8), seems more realistic.

Approximately 30% and 40% of women receiving antiepileptic drug treatment in the age of 20 to 40 years are being treated with valproate or carbamazepine, respectively (10). Therefore, about 100 patients treated with valproate could be expected within a period of 4 to 5 years. With the estimated risk at that time of 1-2% for neural tube defects after valproate exposure, an absence of cases within this period was conceivable and we realized that this study could only provide interesting data over an extended period of time. To give it a serious chance to proceed over such a long period, we preferred a study design with as little intervention and inconvenience as possible, both for the patients as well as the personnel of the outpatient clinic. Inevitably, by this approach some disadvantages have been introduced.

First, intake and amniocentesis appointments were not specifically scheduled for this study, and therefore blood specimens obtained during these visits were taken randomly within the dosing interval.

Secondly, in a study conducted through an outpatient clinic for prenatal diagnosis the choices of appropriate controls are limited. In general, it remains difficult to prove that antiepileptic drug therapy during pregnancy is the causative factor for the increased occurrence of congenital anomalies. Basically two methods have been utilized to evaluate teratogenicity of antiepileptic drugs, *i.e.* by comparison of malformation prevalences among treated with untreated epileptic women or with the general population. With both approaches, consistent evidence has been accumulated of an association between antiepileptic drug therapy and an increased risk for congenital malformations. Although, this consistency might suggest a causal relationship, it may also reflect consistency in confounding factors, which are related to both the treatment and pregnancy outcome. Untreated maternal epilepsy is likely to have a different grade of severity, and non-epileptic controls do not share epilepsy characteristics. We therefore have chosen to make comparisons between women with epilepsy who receive different antiepileptic drugs, and to correlate outcome within medication groups with drug doses and drug levels. Moreover, since there is general agreement that most women require continuation of treatment during pregnancy, at present the crucial question in relation to congenital anomalies and unavoidable drug use is whether the risk of a particular treatment is higher than those conferred by alternative therapies.

2.1.3 Applications of ethical standards

The study was approved by the Medical Ethics Committee of the Erasmus University Rotterdam and the University Hospital Rotterdam Dijkzigt, based on a detailed protocol. The risk of neural tube defects is estimated at 1 to 2% after valproate exposure (11) and 1% after carbamazepine exposure (12), which is 10 to 20 times the risk in the general population and similar to the recurrence risk for parents with a previous child with neural tube defects. Therefore, use of valproate and carbamazepine are accepted indications for amniocentesis at 16 to 20 weeks of gestation in the Netherlands (3). Patients on other antiepileptic drugs, with a lower risk estimate for neural tube defects of

0.3-0.4% (13), which is still several times higher than the risk in the general background population, were only offered an amniocentesis on the patient's own request. Otherwise only a structural ultrasound examination was performed at 20 weeks of gestation. Thus, amniocentesis was always performed primarily for medical, diagnostic procedures. Additional procedures required for this study were the collection of 24 h urine during the day before amniocentesis and the collection of 10 ml venous blood for serum preparation at intake and immediately before amniocentesis.

2.1.4 Application of informed consent

At the first visit of the patient to the out-patient clinic the aim of the study was explained and written information was handed out. The woman was ensured to continue her medication as usual and not to make any changes without consulting her neurologist. Furthermore we asked her to inform us if changes in medication occurred during the period between intake and amniocentesis. Approval was obtained to request medical information from her neurologist and referring obstetrician. Finally, we explained she was free to refuse or withdraw from the study at any time.

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THE RISK OF SPINA BIFIDA APERTA AFTER FIRST-TRIMESTER EXPOSURE TO VALPROATE IN A PRENATAL COHORT

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Abstract

Use of antiepileptic drugs during pregnancy is associated with an increased risk of congenital malformations. Spina bifida aperta has been linked specifically to valproate (estimated risk 1 to 2%). The actual risk, the exclusive association of valproate with spina bifida and not anencephaly, and the precise causative relation remains matter of discussion. A prospective cohort study of pregnant epileptic women with epilepsy receiving antiepileptic drugs and referred for prenatal diagnosis before week 22 of gestation was conducted, with follow-up to 3 months after birth. Pregnancies (291 singleton and 6 twin) in 261 women were evaluated. The prevalence of anomalies after exposure to any antiepileptic drug was 6.9%. For fetuses exposed to valproate, the prevalence was 9.4%, including 6 cases of spina bifida, two of which were in monozygotic twins (giving a prevalence rate of 6.3%, or 5.4%, if twins counted as one). Spina bifida was associated with a significantly higher average daily dose of valproate as compared with pregnancies with normal outcome (1,640 $\pm$ 136 $mg.d^{-1}$ vs 941 $\pm$ 48 $mg.d^{-1}$, $p=0.0001$). No relation was observed between the occurrence of spina bifida and type of maternal seizure or epilepsy, family history of epilepsy or neural tube defects, or medical history. From these results we suggest that when the use of valproate during pregnancy cannot be avoided, the teratogenic risk might be diminished by reduction of the daily dose.

2.2.1 Introduction

Valproate is one of the few commonly used drugs identified during the last decade as a potential human teratogen, inducing spina bifida aperta. An estimated risk of 1 to 2% for spina bifida with maternal use of valproate was made on the basis of retrospective studies (1-3) and a single review of a large number of small prospective cohort studies, each dealing with selected patient populations (4). This increased risk of neural tube defects associated with valproate use during pregnancy became a reason to offer prenatal diagnosis for open neural tube defects by α-fetoprotein determination in amniotic fluid at approximately week 16 of gestation and fetal ultrasound examination (3).

Despite previous findings, several questions around valproate related teratogenesis in humans remain unanswered (5). For example, what is the relevance of a family history found positive for epilepsy or neural tube defects and what is the role of genetic factors related to the maternal epilepsy or seizures during pregnancy?

Whether the association between valproate and neural tube defects is dose dependent - one of the primary criteria applied to the determination of the teratogenicity of any agent - also remains unclear. Studies in mice indicate that the administration of valproate during organogenesis results in a dose-dependent increase of neural tube defects (exencephaly) and that a particular threshold level must be reached before such defects are seen (6, 7). Until now, no data confirming or excluding this dose-dependency in humans has been available.

Another unresolved question deals with why maternal valproate use in humans was reported in association with spina bifida only and not with anencephaly, whereas in rodents the induction of neural tube defects other than exencephaly with valproate has been virtually impossible. Is this finding in humans the result of selective ascertainment, or is it indicative of a specific teratogenic activity of valproate in human that is directed to the caudal neural tube region only?

To delineate the precise relationship between maternal valproate use and birth defects, we initiated a prospective cohort study in the outpatient clinic for prenatal diagnosis of the University Hospital Rotterdam Dijkzigt.

2.2.2 Patients and methods

The protocol was approved by the Medical Ethics Committee of Erasmus University Rotterdam/University Hospital Rotterdam Dijkzigt. The study population consisted of pregnant women with epilepsy using antiepileptic drugs. Subjects were referred to the outpatient clinic for prenatal diagnosis of neural tube defects (without prior knowledge of the fetal condition) and other major malformations by amniocentesis at 16 to 18 weeks and/or structural ultrasound examination at 18 to 22 weeks.

After the women gave informed consent, they completed a standardized questionnaire, with questions on maternal and paternal age, maternal height, weight before pregnancy and upon entering the study, duration of epilepsy, seizure frequency before and during the first trimester of pregnancy, occurrence of seizures with cyanosis, drug regimen (preparation, dose and dosing schedule), obstetric history, complications during pregnancy and other chronic or intercurrent maternal illnesses, medications or exposures. A standardized family history was taken concerning the presence or absence of epilepsy, chronic or hereditary diseases, malformations and mental retardation in first and

second degree relatives of the parents as well as in more remote relatives.

The participants were assured that the medication should be continued as prescribed by the neurologist and were asked to report at the time of amniocentesis whether any changes had been made in medication since their intake into the study.

In the analysis of a dose-outcome relationship, the doses used during the first 8 weeks of pregnancy were considered. More precise information about the underlying epilepsy and obstetric history, as well as about complications and outcome of the present pregnancy was obtained from the neurologist and obstetrician in charge. Epilepsies and seizures were classified according to the International Classification of Epilepsies, Epileptic Syndromes and Related Seizure Disorders of the International League against Epilepsy (8). The seizure-free period before pregnancy and the occurrence of generalized seizures during pregnancy were used as indicators of the severity of the underlying epilepsy. Gestational age was calculated from the first day of the last menstrual period and from ultrasonic measurements of crown-rump length or biparietal diameter. Infants whose birth weight or head circumference below the third centile for duration of pregnancy were categorized as small for gestational age or microcephalic, respectively (9). Only major congenital abnormalities or malformations diagnosed within the first 3 months of life were included in the analysis. Excluded were structurally unbalanced chromosomal abnormalities for which one of the parents was a carrier, and monogenic disorders with mendelian inheritance that were not caused by a *de novo* mutation. After delivery, information was obtained about any significant change in medication and the occurrence of generalized tonic-clonic seizures or status epilepticus during the second and third trimesters of pregnancy, the mode of delivery, and the infant's health.

Chi-square statistics for categorial data and *t*-test statistics (mean $\pm$ SEM) for continuous data were used for comparison between groups. 95% confidence limits (CIs) were calculated for proportions.

Table 2.2.1 Use of antiepileptic drugs during the first trimester of pregnancy in the cohort compared with that in the general population of women with epilepsy 20 to 39 years old

Drug		Cohort				General population*
		Mono	Poly	Total	%	%
Valproate	(VPA)	60	32	92	31	32
Carbamazepine	(CBZ)	114	45	159	54	37
Phenytoin	(DPH)	28	19	47	16	14
Phenobarbital	(PHB)	18	26	44	15	
Primidone	(PMD)	0	4	4	1	
Ethosuximide	(ESX)	2	5	7	2	
Diazepam	(DZP)	1	1	2	(0.7)	
Clonazepam	(CLP)	0	13	13	4	
Clobazam	(CLM)	2	6	8	3	
Acetozolamide	(AZA)	1	0	1	(0.3)	
Chlorazepate		0	1	1	(0.3)	
Total		226	71	297		

* Prescription of antiepileptic drugs for epilepsy to women 20 to 39 years old (averages over the years 1985 to 1989 (10).

2.2.3 Results

From November 1, 1985 until July 1, 1990, 297 pregnancies involving 303 fetuses from 261 different women were investigated. Table 2.2.1 presents the use of the various antiepileptic drugs in the cohort *vs.* the general population of Dutch women with epilepsy,

Table 2.2.2 Congenital anomalies and antiepileptic drugs used

Nr.	Type of congenital anomalies	DPH	PHB	PMD	CBZ	VPA	CLP	CLM	ACA	♂/♀
1.	SBA & H	-	-	-	-	1700	-	-	-	♂
2.	SBA (donor*) & H	-	-	-	-	1800	-	-	-	♂
3.	SBA (acceptor*) & H	-	-	-	-	1800	-	-	-	♂
4.	SBA	-	-	-	-	1200	-	-	-	♂
5.	SBA & H	-	-	-	-	2000	-	-	-	♀
6.	SBA (meningocele) & H	-	-	-	600	1500	-	-	-	♀
7.	Hypospadia, hydrocephalus, cleft lip and palate	-	-	-	200	-	-	-	-	♂
8.	Cleft lip	-	150	-	-	-	-	-	-	♂
9.	Cleft palate (soft total, hard 1/3)	-	-	-	800	1200	-	-	-	♀
10.	Congenital heart defects VSD, ASD, PS, ODB	-	150	-	-	-	-	-	-	♀
11.	Club foot left	-	-	-	600	-	-	-	-	♀
12.	Club foot right	-	-	-	-	1000	-	-	-	♂
13.	Microcephaly (<-3 SD)	-	-	750	600	-	-	30	-	♀
14.	Inguinal hernia	-	-	-	-	750	-	-	-	♂
15.	VSD, †SASDS, radiusaplasia left hemivertebrae (1,3 & 5 chest)	-	150	-	-	-	-	-	-	♀
16.	Hypospadia, ptosis (S.Horner)	-	-	-	600	-	-	-	-	♂
17.	Hypertrophic pylorusstenosis	-	-	-	400	-	-	-	-	♂
18.	‡PMR, seizures, strabismus divergens	300	30	-	-	-	-	-	-	♀
19.	§IgG-deficiency, epilepsy, heart murmur	-	-	-	400	-	2	-	-	♂
20.	§IgA-deficiency, thymushypoplasia	-	-	-	400	-	2	-	-	♂
21.	Chylothorax & persistent fetal circulation	-	-	-	-	-	-	-	250	♀

DPH = phenytoin
PHB = phenobarbital
PMD = primidone
CBZ = carbamazepine
VPA = valproic acid
CLP = clonazepam
CLM = clobazam
ACA = acetazolamide

SBA = spina bifida aperta,
H = hydrocephalus, based on dilated ventricles at ultrasound examination
VSD = ventricular septal defect,
ASD = atrial septal defect,
ODB = open ductus botalli,
PS = pulmonary artery stenosis
SASDS = subclavian artery supply dysruption sequence
PMR = psychomotor retardation

* Twin-twin transfusion, donor and acceptor
† SASDS, without previous chorionic villus sampling. Final diagnosis: Möbius Poland like anomaly
‡ Mother known to be alcohol abuser
§ Sibs, no known immune deficiency syndrome, parents were immune competent and not consanguineous, and family history was negative for immune deficiency.

aged 20 to 39 years (10). In this cohort of 297 women, carbamazepine was the most frequently used drug (159), followed by valproate (92). Monotherapy was prescribed in 76% (226) of all pregnancies and in 65% (60 of 92) of valproate-exposed pregnancies. From the combinations of two drugs, that of carbamazepine plus valproate was the most frequently used antiepileptic drug combination (14 of 71, 20%).

No amniocentesis was performed in 30 pregnancies because of late referral (n=21), objections by the woman (n=7) or obstetric reasons (n=2).

Two fetuses died within 1 week of amniocentesis. One death was associated with intrauterine infection and the other with spontaneous abortion of a fetus of a twin pregnancy. One stillbirth occurred after placental abruption. Two infants delivered prematurely died during the neonatal period, of respiratory failure.

The number of congenital abnormalities in infants (and fetuses) observed in this study was 21 (table 2.2.2), with an overall prevalence of 6.9% (21/303, 95% CI, 4.0% to 9.8%). For each of the major antiepileptic drugs, the prevalence of defects was as follows: phenytoin 2.1% (1/47), phenobarbital 9.1% (4/44), carbamazepine 5.7% (9/159) and valproate 9.4% (9/96). Amongst the valproate exposed fetuses, 6, including 1 monozygotic pair of twins, displayed an open (lumbo) sacral spina bifida (6/96, 6.3%, 95% CI 1.4% to 11.1% or twins counted as one 5/92, 5.4%, 95% CI 0.8% to 10.1%). Compared with the other antiepileptic drugs, with which no cases of neural tube defects were seen, this association is highly significant (p=0.0003) (table 2.2.3). All 5 pregnancies were terminated. Autopsies of the 4 singleton fetuses confirmed the diagnosis. Permission for postmortem examination of the affected pair of twins was denied, but visual inspection confirmed the presence of a sacral meningomyelocele in both.

Table 2.2.3 Spina bifida aperta in fetuses (pregnancies) of epileptic women exposed to valproate compared to epileptic mothers using other antiepileptic drugs

	no. of fetuses (no. of pregnancies)		
	SBA★	Other	Total
Valproate	6 (5)	90 (87)	96 (92)
Other antiepileptic drugs	0	207 (205)	207 (205)
Total	6 (5)	297 (292)	303 (297)

★ SBA = spina bifida aperta, Chi-square=13.2, p=0.0003

No significant differences were seen among the 5 successive years of the study with respect to proportion and absolute numbers of women receiving valproate and the mean daily dose of valproate. No cases of anencephaly were observed in this cohort, whereas in the same centre during the same period, anencephaly and spina bifida aperta were diagnosed in equal numbers in the fetuses of women referred for prenatal diagnosis

of neural tube defects because of other risk factors.

In pregnancies with fetal spina bifida, significantly higher devided doses (*i.e.* dose per administration) and total daily doses were used, whereas the number of doses per day was not different from pregnancies with normal outcome. With daily doses of 1,000 $mg.d^{-1}$ or less no spina bifida was observed (0/54), and with doses from 1,000 to 1,500 $mg.d^{-1}$ 2 cases (2/30, 6.7%); in the remaining 3 affected pregnancies, the doses were more than 1,500 $mg.d^{-1}$ (3/8, 37.5%). Also the levels of valproate in maternal serum were significantly higher in women with spina bifida outcome (table 2.2.4).

Table 2.2.4 Mean daily valproate dose and number of gifts according to pregnancy outcome

	Pregnancy outcome			*t*-test
	SBA n=5	Other defects n=3	Normal outcome n=84	p_1 = SBA *vs* normal p_2 = Other *vs* normal
Daily dose (mg)				
Mean±SEM	1640 ± 136	983 ± 130	941 ± 48	$p_1 = 0.001$
range	200 - 2000	750 - 1200	100 - 2400	$p_2 = 0.869$
Daily dose ($mg.kg^{-1}$)				
Mean±SEM	25 ± 2	14 ± 1	14 ± 0	$p_1 < 0.001$
range	22 - 32	11 - 15	1 - 32	$p_2 = 0.902$
Valproate concentration in maternal serum ($\mu g.ml^{-1}$)	73.4 ± 25.0		43.9 ± 21.6	$p^* = 0.023$
Peakdose per administration (mg)				
Mean±SEM	650 ± 124	350 ± 76	384 ± 19	$p_1 = 0.002$
range	300 - 1000	250 - 500	100 - 1125	$p_2 = 0.740$
No. of doses per day				
Mean±SEM	2.8 ± 0.4	3.0 ± 0.6	2.5 ± 0.1	$p_1 = 0.497$
range	2 - 4	2 - 4	1 - 4	$p_2 = 0.357$

* = p-value of Mann-Whitney U-test

Women receiving valproate differed from women receiving other antiepileptic drugs with regard to the type of epilepsy and seizures. Valproate was prescribed more frequently for primary generalized epilepsy, whereas other antiepileptic drugs, mainly carbamazepine, were associated more with partial epilepsy. When data were analyzed according to type of epilepsy or seizure type, however, the risk for spina bifida risk associated with valproate remained essentially unchanged. None of the women carrying a fetus with spina bifida experienced seizures during the first trimester of pregnancy (table 2.2.5).

Women treated with valproate had more relatives with epilepsy than did women receiving other antiepileptic drugs. In 4 of the 5 women with fetuses with spina bifida, the family history was negative for epilepsy, except for the maternal epilepsy itself. For the remaining case of spina bifida, the family history was positive for epilepsy on both sides (the maternal grandfather and a brother of the father). For each case of spina bifida, both the paternal and maternal family histories were negative for neural tube defects.

Furthermore, for all 5 pregnancies with spina bifida outcome, the obstetric history concerning neural tube defects in previous pregnancies was negative, regardless of whether fetal antiepileptic drug exposure was similar, different, or absent.

2.2.4 Discussion

In this prospective cohort 6.3% (95% CI 1.4% to 11.1%) of fetuses, or 5.4% of pregnancies (0.8% to 10.1%) with valproate exposure had (lumbo)sacral spina bifida aperta. In previous studies, the risk of spina bifida with valproate was estimated at 1 to 2% (2, 4). It could be demonstrated that a significantly higher dose of valproate was used in pregnancies resulting in a child with spina bifida. These findings provide strong evidence for a causal association between maternal use of valproate and fetal spina bifida.

In our study, spina bifida was consistently associated with valproate use and not with the other antiepileptic drugs. Only one infant had been exposed to valproate plus carbamazepine. Maternal carbamazepine monotherapy was recently also connected to fetal spina bifida, but with a lower risk (11) than with valproate. In this cohort, no case of spina bifida was observed among the 114 pregnancies exposed to carbamazepine alone.

Pregnancies with negative outcome were unlikely to have been selectively ascertained. The estimated participation rate for prenatal diagnosis of women receiving antiepileptic drugs in this study was over 50%, a rate even higher than that achieved (44%) for advanced maternal age in this region (12). The distribution of antiepileptic drugs used in this cohort was not essentially different from that in a random sample of the general population of women in the age group of 20 to 39 years of age. Only pregnancies for which there was no prior knowledge of the fetal condition were included in the study.

The Netherlands has no program for general maternal screening for serum α-fetoprotein. In the area from which pregnant women were referred, no such screenings were carried out on a hospital basis. Women participating in the study did not have more additional indications for prenatal diagnosis than expected. The proportion of mothers of advanced maternal age (27/297, 9%) was not different from that in the general Dutch population (8%).

Theoretically, prenatal diagnosis might increase the ascertainment of neural tube defects if a considerable proportion of pregnancies with fetal spina bifida would abort spontaneously between 16 and 24 weeks without prenatal diagnostic procedures, and such a defect would escape diagnosis and/or registration. No change in the frequency of neural tube defects occurred after the introduction of prenatal diagnosis of neural tube defects, however, and in the general population, only approximately 1% of infants with a neural tube defect are born before term (13). Furthermore, there is no reason to suspect that especially the valproate-induced spina bifida, in particular, is associated with a higher risk of fetal loss between 16 and 24 weeks, as compared with that for spontaneously occurring spina bifida. In the present study, none of the fetuses with spina bifida had other anomalies known to affect fetal survival.

The distribution of spina bifida cases over the period of the study also was

Table 2.2.5 Epilepsy and seizure characteristics of mothers exposed to valproate (VPA) during pregnancy compared to mothers exposed to other antiepileptic drugs (AEDs)

	VPA (SBA)★ (n=92)			Other AEDs (n=205)		Total (n=297)		†p-value
	nr.		%	nr.	%	nr.	%	
Type of epilepsy								<0.001
- Primary gen.	57	(3)	62.0%	63	30.7%	120	40.4%	
- Partial	28	(2)	30.4%	115	56.1%	143	48.1%	
- Unclassifiable	3		3.3%	8	3.9%	11	3.7%	
- Unknown	4		4.3%	19	9.3%	23	7.7%	
Seizure types‡								<0.001
- Simple focal	1		1.1%	6	2.9%	7	2.4%	
- Complex focal	3		3.3%	19	9.3%	22	7.4%	
- Focal, sec. gen. tonic-clonic	23	(2)	25.0%	92	44.9%	115	38.7%	
- Primarily gen. tonic-clonic	54	(3)	58.7%	61	29.8%	115	38.7%	
- Absence	3		3.3%	2	9.8%	5	1.7%	
- Unclassifiable§	3		3.3%	3	1.5%	6	2.0%	
- Unknown	5		5.4%	22	10.7%	27	9.1%	
Etiology								<0.001
- Unknown etiology	75	(5)	81.5%	151	73.7%	226	76.1%	
- Perinatal	3		3.3%	11	5.4%	14	4.7%	
- Postencephalic	2		2.2%	12	5.9%	14	4.7%	
- Posttraumatic	1		1.1%	22	10.7%	23	7.7%	
- Familial	10		10.9%	5	2.4%	15	5.1%	
- Unknown	1		1.1%	4	2.0%	5	1.6%	
Age at first seizure								0.003
- 0 years	0		0%	13	6.3%	13	4.4%	
- 1-10 years	25	(2)	27.2%	43	21.0%	68	22.9%	
- 11-20 years	54	(3)	58.7%	96	46.8%	150	50.5%	
- 21-30 years	9		9.8%	37	18.0%	46	15.5%	
- 31-40 years	1		1.1%	4	2.0%	5	1.7%	
- Unknown	3		3.3%	12	5.9%	15	5.1%	
Early onset (<20)	78	(5)	87.6%	141	73.1%	219	77.7%	0.004
Late onset (≥20)	11		12.4%	52	26.9%	63	22.3%	
Duration of epilepsy before present pregnancy								0.008
- 20 or more years	22		23.9%	55	26.8%	77	25.9%	
- 10 to 20 years	46	(4)	50.0%	64	31.2%	110	37.0%	
- 10 or less years	21	(1)	22.8%	74	36.1%	95	32.0%	
- Unknown	3		3.3%	12	5.9%	15	5.1%	
Seizure free period before present pregnancy								0.030
- less than 1 year	11	(1)	12.0%	43	21.0%	54	18.2%	
- 1 to 5 years	58	(3)	63.0%	103	50.2%	161	54.2%	
- more than 5 years	17	(1)	18.5%	21	10.2%	38	12.8%	
- Unknown	6		6.5%	38	18.5%	44	14.8%	
Generalized seizures during pregnancy¶			(n=75)		(n=150)		(n=225)	
- any time								0.103
yes	19		25.3%	54	36.0%	73	32.4%	
no	56		74.7%	96	64.0%	152	67.6%	
- 1st trimester								0.183
yes	11		14.7%	33	22.0%	44	19.6%	
no	64	(5)	85.3%	117	78.0%	181	80.4%	
- 2nd trimester								0.041
yes	8		10.7%	32	21.3%	40	17.8%	
no	67		89.3%	118	78.7%	185	82.2%	
- 3rd trimester			(n=69)		(n=148)		(n=217)	0.227
yes	9		13.0%	29	19.6%	38	17.5%	
no	60		87.0%	119	80.4%	179	82.5%	

★ Number in parentheses are for mothers carrying a fetus affected with spina bifida aperta.
† The "unknown" category is excluded from the analyses.
‡ Hierarchic classification, *i.e.* complex focal seizures in combination with focal seizures, evolving to generalized tonic-clonic seizures are classified as tonic-clonic seizures.
§ Including myoclonic seizures and akinetic seizures.
¶ Only patients who were experiencing generalized seizures before pregnancy included.

analyzed, since 3 cases occurred in the spring of 1990. Within the prospectively collected dataset, no relevant risk factor other than valproate use itself was identified in any of the cases. No geographic clustering of maternal domiciles existed, and the women did not report the birth of other babies with spina bifida within their neighbourhood during the months following prenatal diagnosis of spina bifida in their fetuses.

To exclude a change in the composition of the marketed valproate preparations, we retrieved samples of the preparations taken during early pregnancy by 3 of the 5 women whose fetuses had spina bifida. The samples were analyzed by one of the manufacturers of valproate and the composition of each was reported as falling within the limits of the specifications. Furthermore, the manufacturers reported that they had reviewed the entire process of production, from the materials used for synthesis up to delivery of the drug to the pharmacy, and found that no changes had been introduced during the relevant period.

Previous studies did not show any association between anencephaly and maternal valproate use. Underascertainment, incomplete follow-up, and underdiagnosis have been put forward as explanations for the observed lack of an association. The present study, however, was prospective, with early ascertainment and 100% follow-up, including that of immature or premature deliveries, intrauterine deaths, neonatal deaths, and terminations of pregnancy. The absence of anencephaly cannot be explained by early diagnosis and termination of pregnancy before referral to the centre, since spina bifida and anencephaly were diagnosed in equal numbers (4/4) in pregnancies referred for prenatal diagnosis because of a genetic risk of recurrence or maternal diabetes mellitus.

Neural tube defects associated with maternal valproate use are potentially preventable disorders, if medication is reconsidered before pregnancy. The physician making this choice should take into account the therapeutic benefits and the specific teratogenic risks of various regimens, as well as the attitude of the woman and her partner toward prenatal diagnosis and termination of pregnancy. If valproate use during pregnancy is unavoidable, offering prenatal diagnosis is mandatory. In view of the dose-dependent effect observed in the present study, as well as in experimental animal (7), we recommend the use of the lowest possible daily dose to reduce the teratogenic risk. Assuming that high serum levels of valproate in the mother increase the risk, we also advise that the daily dose be administered in at least three single doses per day.

2.2.5 References

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SPECTRUM OF NEURAL TUBE DEFECTS IN 34 INFANTS PRENATALLY EXPOSED TO ANTIEPILEPTIC DRUGS

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Abstract

We analyzed the spectrum of neural tube defects associated with maternal exposure to antiepileptic drugs and the possible contribution of familial and genetic factors to epilepsy or neural tube defects. No specific association with maternal family history of neural tube defects or epilepsy was seen. The ration of spina bifida to anencephaly (33:1) suggested a specific association with caudal defects. Hydrocephaly was documented in at least 21 cases. Other midline defects, all associated with valproate, were hypospadias (2), hyperteloriSm (2), partial agenesis of corpus callosum, agenesis of septum pellucidum with lissencephaly of medial sides of occipital lobes, Dandy-Walker anomaly, and ventricular septal defect. This study shows that most neural tube defects following maternal valproate use are severe open defects. They are frequently complicated by hydrocephaly and other midline defects. Prenatal diagnosis is possible.

2.3.1 Introduction

Maternal epilepsy and use of antiepileptic drugs during pregnancy are associated with a twofold to threefold increase in congenital anomalies in offspring (1-3). The classical antiepileptic drugs - such as barbiturates, primidone, and phenytoin - are associated predominantly with heart defects and cleft lip and palate, especially when used in combination (4-6). The more recently introduced drugs valproic acid and carbamazepine show relatively stronger associations with spina bifida aperta (1 to 2% and 0.5 to 1%, respectively) and possibly with hypospadias (7-11). It remained unclear why valproic acid was associated with spina bifida aperta but not with anencephaly (12), and whether this was a result of bias in case ascertainment or indicated a difference between the target susceptibility of the causal and rostral neural tube or a difference in etiology of spina bifida and anencephaly in general.

A positive family history of neural-tube defects has been reported in a number of published cases, and this finding has frequently been used as an argument against a causal relation between valproate exposure and spina bifida, and for an etiologic link - through inheritance or by transplacental mechanisms - between maternal epilepsy and fetal neural-tube defects.

It was suggested that the valproate-induced neural-tube defects were in general a milder type of closed lipomeningoceles rather than open defects with severe neurologic dysfunction. Implicitly, this would raise doubt about indication, as well as the reliability, of prenatal diagnosis early in second trimester of pregnancy. In 1984 the Dutch media fully informed the Dutch professional (13) and lay public about the observed association between maternal valproate use and spina bifida and the need for prenatal monitoring. It was therefore of interest to evaluate whether this publicity had influence on the proportion of cases that were prenatally diagnosed.

To address some of these questions, we analyzed all cases of any neural-tube defect known to us through six different primary sources in The Netherlands.

2.3.2 Methods

All possible sources were used to ascertain cases of neural-tube defects following maternal antiepileptic drug use. Affected infants had to have been born after the year 1971, when valproate was released for prescription in The Netherlands (carbamazepine had been introduced 3 years earlier).

Specialized outpatient clinics contributed cases during the entire period of the prospective study. Patients attending these outpatient clinics represent approximately 7% of Dutch population with epilepsy and are usually referred because of severe intractable epilepsy, seizures that are difficult to diagnose, or a need for special psychosocial attention (11).

The Rotterdam prospective prenatal cohort, assembled from 1985 through 1991, was comprised of women referred for prenatal diagnosis (*i.e.* before the 20th week of

gestation) because of antiepileptic drugs use. Maternal valproate use has been a nationally accepted indication for prenatal diagnosis by amniotic fluid analysis and fetal ultrasound examination since 1984. Maternal carbamazepine use had been an indication for similar tests since 1985. The Rotterdam cohort covers a region of about 40,000 births per year, and approximately 50% of women receiving antiepileptic drugs are actually referred. All women receiving antiepileptic drugs other than valproate or carbamazepine undergo ultrasound examination, whereas only women who insist on amniocentesis, or in whom there are additional indications for α-fetoprotein analysis of amniotic fluid or chromosome analysis of amniotic cells, undergo this invasive diagnostic procedure.

In 1984, 20 teams involved in treatment of spina bifida were asked to report on patients with spina bifida exposed *in utero* to any antiepileptic drug and born after 1971. Four teams responded and contributed five cases; a sixth case was contributed later (8,13,14).

Since 1972, single cases have been brought directly to the attention of one of the authors or to the Medical Service Department of Sanofi Holland or the Dutch Centre for Monitoring Adverse Reactions of Drugs. Three of the eight regional clinical genetics departments in The Netherlands carried out a more-or-less systematic survey of their case experience, including obstetric histories obtained during genetic counselling, and each contributed one case.

The EUROCAT Birth Defects Registry in Groningen started in 1981 and today covers approximately 19,000 births per year in the north-eastern region of The Netherlands. It registers infants with congenital malformations diagnosed during the first year of life, including fetuses with malformations detected prenatally followed by termination of pregnancy. The information registered concerns the diagnosis, as well as maternal exposures to drugs, chemicals, and other potential environmental risk factors.

After initial ascertainment by one of these sources, additional information on some cases became available from another source. This information was then used to complete and check the accuracy of data. Only the primary sources are summarized in table 2.3.1.

Affected infants were thought to have hydrocephaly when any of the following signs was present: a lemon sign or ventricular dilatation on fetal ultrasound examination; hydrocephaly observed at post mortem examination; clinical diagnosis of hydrocephaly by neurologist or neurosurgeon; presence of ventriculoatrial or ventriculoperitoneal drainage.

Epilepsy was classified according to the International Classification of Epilepsies and Seizures (15) if sufficient reliable information was obtained. Zygosity of twins was determined by inspection of fetal membranes and analysis of DNA polymorphisms. The present report, with 24 new cases, can be considered as an extension of the first 10 cases reported in 1984 (8,13,14).

Table 2.3.1 Sources of neural tube defect cases associated with prenatal antiepileptic drug exposure (The Netherlands, last update July 15, 1991)

Source	Total No. of cases	Exposure VPA	CBZ	VPA + CBZ	VPA + OCB	VPA + PHB	PHB
Prospective cohorts							
-Epilepsy outpatient clinics	7	*3	†3	-	‡1	-	-
-Rotterdam prenatal diagnosis clinic	7	6	-	1	-	-	-
Spina bifida teams	6	4	-	§2	-	-	-
Single case reports	8	6	2	-	-	-	-
Clinical genetics departments	3	2	-	-	-	1	-
Birth defects registry Groningen	3	1	1	-	-	-	1
Total	34	22	6	3	1	1	1

VPA = valproic acid; CBZ = carbamazepine; OCB = oxcarbamazepine; PHB = phenobarbital
* Valproate (2,000 mg.d^{-1}) + chlorodiazepoxide (20 mg.d^{-1}) + acetazolamide (500 mg.d^{-1}) (n=1); Valproate (1,500 mg.d^{-1}) + clonazepam (3 mg.d^{-1}) (n=1).
† Carbamazepine (400 mg.d^{-1}) + oral contraceptive failure (n=1).
‡ Valproate (1,700 mg.d^{-1}) + oxcarbamazepine (3,000 mg.d^{-1}) + clobazam (20 mg.d^{-1})(n=1)
§ Valproate (1,800 mg.d^{-1}) + carbamazepine (600 mg.d^{-1}) + phenytoin (375 mg.d^{-1}) + ethosuximide (750 mg.d^{-1}) + oral contraceptive failure

Table 2.3.2 Type of neural tube defect after maternal antiepileptic drug use

Source	Total No. of cases	Exposure VPA	CBZ	VPA + CBZ	VPA + OCB	VPA + PHB	PHB
Open defects							
anencephaly	1	-	-	-	-	-	1
SBA-TL	1	1	-	-	-	-	-
SBA-L	4	2	-	1	*1	-	-
SBA-LS	16	†13	1	‡1	-	1	-
SBA-S	7	3	§3	1	-	-	-
SBA-Unspecified	2	1	1	-	-	-	-
Closed spinal defects	3	‖2	1	-	-	-	-
Total	34	22	6	3	1	1	1
Total with known hydrocephaly	21	16	3	2	-	-	-

VPA = valproic acid; CBZ = carbamazepine ; OCB = oxcarbamazepine; PHB = phenobarbital.
SBA = spina bifida aperta; TL = thoracolumbal; L = lumbar; LS = lumbosacral; S = sacral
* Valproate (1,700 mg.d^{-1}) + oxcarbamazepine (3,000 mg.d^{-1}) + clobazam (20 mg.d^{-1}) (n=1).
† Valproate (2,000 mg.d^{-1}) + chlorodiazepoxide (20 mg.d^{-1}) + acetazolamide (500 mg.d^{-1}) (n=1).
‡ Valproate (1,800 mg.d^{-1}) + carbamazepine (600 mg.d^{-1}) + phenytoin (375 mg.d^{-1}) + ethosuximide (750 mg.d^{-1}) + oral contraceptive failure
§ Carbamazepine (400 mg.d^{-1}) + oral contraceptive failure (n=1).
‖ Valproate (1,500 mg.d^{-1}) + clonazepam (3 mg.d^{-1}) (n=1).

2.3.3 Results

Thirty-four cases of neural-tube defects were identified through six different sources (table 2.3.1). Approximately two-thirds of the identified infants had been exposed to valproate, in the majority of cases as a single drug. The second most frequently used drug was carbamazepine. In only one case was the infant exposed to a drug (phenobarbital) other than valproate and/or carbamazepine. This case was ascertained through the EUROCAT registry. Two sibling fetuses whose mother used phenobarbital during both pregnancies were excluded from the survey, because it was not certain whether the abnormalities were indeed neural-tube defects. In two cases, pregnancy had occurred despite the mother's use of oral contraception. Both mothers had used carbamazepine, a compound known to induce steroid metabolism (16). Almost all cases had lumbar, sacral, or lumbosacral spina bifida (table 2.3.2). In only one case of thoracolumbar spina bifida aperta had the fetus been exposed to valproate. The single case of anencephaly was also the only case not associated with valproate or carbamazepine, but with phenobarbital. In two cases, multiple dysmorphic features were reported that were reminiscent of earlier delineations of fetal valproate syndromes (17, 18).

In 28 of 34 cases, information about the maternal disease was sufficient for classification of epilepsy. Not all cases of valproate exposure were associated with primary generalized epilepsy in the mother; in at least five cases, valproate was used for partial epilepsy (table 2.3.3).

A positive family history of neural-tube defects was present in six

Table 2.3.3 Neural tube defects after maternal antiepileptic drug exposure: relation between type of maternal epilepsy and maternal drug treatment

Type of epilepsy	VPA	CBZ	VPA + CBZ/OCB	Other
Primary generalized	15	1	3	-
Partial	5	3	1	-
Unknown/unclassified	3	2	-	1

VPA = valproic acid; CBZ = carbamazepine; OCB = oxcarbamazepine

Table 2.3.4 Neural tube defects after maternal antiepileptic drugs exposure: relation with type of maternal epilepsy and family history of neural tube defect

	Family history of neural tube defects				
	negative	positive			
Type of maternal epilepsy		maternal family	paternal family	both families	sibs exp. + affected
Primary generalized	12	-	2*	1†	4‡
Partial	6	1§	-	-	2§
Unknown/ unclassified	4	1¶	1#	-	-

* Paternal relative had spina bifida (case no. 6); brother of the father had died of spina bifida (case no. 8).
† Sister of mother of the father and child of sister of father of the mother had spina bifida (case no. 18)
‡ Pair of sibs both exposed to 2,400 mg.d^{-1} of valproic acid and both affected (case nos. 3 and 4); pair of monozygotic twins exposed to 1,800 mg.d^{-1} of valproic acid and concordantly affected (cases nos. 28 and 29).
§ Child of sister of the mother had Dandy-Walker anomaly (case no. 33).
‖ Pair of sibs both exposed to 2,000 mg.d^{-1} of valproic acid and both affected (case nos. 30 and 34).
¶ Son of daughter of sister of mother had spina bifida (case no. 24).
# Sister of mother of father had hydrocephaly (case no. 23).

cases: In two cases, maternal relatives were involved; in three, paternal relatives; and in one, both maternal and paternal relatives had neural-tube defects (table 2.3.4). Furthermore, two pair of sibs and one pair of monozygotic twins were concordant not only for antiepileptic drug exposure but also for type and localization of spinal defects. Otherwise, family histories were negative for neural-tube defects. A positive family history of neural tube-defects was not associated with a specific type of maternal epilepsy.

A positive family history of epilepsy was obtained for nine cases (table 2.3.5): Five had one or more maternal relatives with epilepsy; two had a father with epilepsy, as well as a maternal relative with epilepsy; one had a father with epilepsy; and one had a paternal relative with epilepsy. No association with a specific type of maternal epilepsy was observed.

Table 2.3.5 Neural tube defects after maternal antiepileptic drugs exposure: relation with type of maternal epilepsy and family history of epilepsy

Type of maternal epilepsy families	Family history of epilepsy: Negative	Positive: maternal family	Positive: paternal family	Positive: both
Primary generalized	14	3*	2†	-
Partial	5	2‡	-	2§
Unknown/unclassified	6	-	-	-

* Sister of the mother and daughter of this sister of the mother had epilepsy (case no. 9); sister and mother of the mother had epilepsy (case no. 11); father of the mother had epilepsy (case nr. 18).
† Father also had epilepsy and was receiving carbamazepine and phenytoin (case no. 15); an aunt of the father had epilepsy (case no. 17)
‡ Sister of the mother had epilepsy (case no. 26); sister of the mother had epilepsy (case no. 33).
§ Mother of the mother had epilepsy (case no. 16) and father also had epilepsy and was receiving valproic acid; son of sister of the mother, as well as the father had epilepsy; the father was receiving phenytoin and chlorazepate (case no. 27).

Before 1984, one case of spina bifida associated with valproate exposure was prenatally diagnosed, but only during the 29th week of pregnancy, when ultrasound examination was performed for obstetric reasons. After 1984, 10 of 15 cases associated with valproate exposure were diagnosed prenatally by amniotic fluid analysis and ultrasound examination, and diagnosis was followed by termination of pregnancy (table 2.3.6). Congenital malformations in addition to the neural-tube defect were reported only in cases of valproate exposure and were predominantly of the midline type (table 2.3.7).

The mean daily dose used by the mothers of infants with spina bifida was 1,530 mg for valproate monotherapy and 1,560 mg for valproate polytherapy (table 2.3.8). Within the Rotterdam prenatal cohort, the mean daily dose of valproate used by mothers who had normal results of prenatal analysis and delivered normal babies was 940 mg (SD $\pm$ 440 mg; n=83). After

Table 2.3.6 Prenatal and postnatal diagnosis of neural tube defects after maternal antiepileptic drugs use, before and after 1984*.

Age at diagnosis	1972-1984		1985-1991	
At birth	11	(14)	3	(7)
Prenatally, confirmed at birth	0	(1)	2	(2)
Prenatally, confirmed at termination of pregnancy	0	(-)	10	(10)

* Numbers represent only cases with open defects after valproic acid exposure; numbers within parentheses also include cases with closed defects and cases exposed to antiepileptic drugs other than valproic acid.

Table 2.3.7 Neural tube defects after maternal valproate use: additional other defects

Midline defects	Other defects
Hypospadias (twice)	Multiple skeletal defects including bilateral radial aplasia
Hypertelorism (twice)	
Partial agenesis of corpus callosum	Bilobar lungs
Agenesis of septum pellucidum with lissencephaly of medial sides of occipital lobes	
Dandy Walker anomaly and ventricular septum defect	

the seven cases from the Rotterdam cohort are excluded, the mean daily dose to which the remaining affected infants in the present survey had been exposed is 1,470 mg (SD ± 580 mg). This provides indirect and independent confirmation that the risk of spina bifida is dose-dependent.

No significant deviation from the expected 1:1 sex ratio was seen, and no differences in maternal age were noted in affected offspring exposed to different medications (table 2.3.8). Birth weight of affected offspring exposed to valproate in combination with other antiepileptic drugs was significantly lower than that of those exposed to valproate monotherapy or carbamazepine monotherapy (table 2.3.8).

Table 2.3.8 Neural tube defects after maternal use of valproate, carbamazepine or both (n=33): daily dose, maternal age, birth weight, and sex ratio

	Monotherapy		Valproate polytherapy (including CBZ)
	CBZ	VPA	
Daily dose (mg)*	n=6	n=19	n=7
Mean ± SD	570 ± 80	1530 ± 530	1560 ± 500
Range	400-600	600-2400	500-2000
Maternal age (yrs)	n=6	n=20	n=7
Mean ± SD	28.8 ± 4.1	27.1 ± 3.6	29.0 ± 5.5
Range	21-37	23-34	22-40
Birth weight (g)*,†	n=5	n=7	n=5
Mean ± SD	3400 ±400	3290 ± 120	2790 ± 360
Range	3050-3950	3120-3420	2280-3170
Sex ratio (♂/♀)*	3/3	10/9	4/3

* A single case missing values for sex, birth weight, and duration of pregnancy.
† Birth weight only of infants born at term.

2.3.4 Discussion

In this study we confirmed the association of valproate use with spina bifida aperta. We did not confirm association of valproate use with anencephaly. In theory, a more selective ascertainment of spina bifida could have taken place, perhaps because of the chronic psychosocial impact of such a handicap since spina bifida is compatible with survival. Handicaps such as anencephaly are usually lethal in the perinatal period. We could have counted more cases of spina bifida, because we diagnosed more of them prenatally and were able to pathologically verify them after termination of pregnancy. Obstetricians could have failed to report cases of anencephaly that were diagnosed by routine ultrasound examination late in the first or very early in the second trimester of pregnancy that led to termination of pregnancy. Mothers might have forgotten to inform their neurologists about the fatal outcome of their short pregnancies.

The chances for the reporting of a handicap of a surviving infant are, of course, greater than for fatal handicaps. We used all possible sources for ascertainment of cases, however, and it is unlikely that all of these would identify only cases of spina bifida. The Rotterdam prenatal cohort study ascertains pregnancies at risk before the 12th week of pregnancy and before the fetal condition is known. After prenatal examination, complete follow-up information on reproductive outcome is obtained. The Birth Defects Registry of Groningen registers approximately equal numbers of cases of anencephaly and spina bifida that are not associated with exposure to antiepileptic drugs.

The three clinical genetics departments also ascertain cases when the obstetric history is taken, which is retrospective but independent of the type of defect.

The previous birth of a child with an open neural-tube defect is a widely accepted indication for genetic counselling and prenatal diagnosis, independent of whether the defect occurred spontaneously or in association with maternal antiepileptic drugs use. There is no reason to believe that, in contrast to the general population of parents with a previous child with a neural-tube defect, parents with such a child born after maternal use of antiepileptic drugs would attend these clinics for subsequent genetic counselling only if the defect is spina bifida and not anencephaly. Also, clinical genetics centres besides the one in Rotterdam identify both types of neural-tube defects equally well by early prenatal diagnosis in women referred primarily because of antiepileptic drugs use.

These three sources - the Rotterdam prenatal cohort, the EUROCAT Birth Defects Registry, and the clinical genetics departments - contributed 11 cases of spinal defects after maternal valproate use and no cases of anencephaly. Although the cohort from the epilepsy outpatient clinics and the series of single-case reports both lack reported cases of anencephaly, this can hardly explain the lack of such cases among 10 reports on neural-tube defects after maternal valproate use. Only six of the 27 cases of spina bifida after maternal valproate use were identified through single-case reports, which are most sensitive to reporting bias.

The specific association of valproate with lumbosacral spina bifida aperta and not with anencephaly suggests that the etiologic factors and pathogenetic mechanism involved in spontaneously occurring spina bifida are different from those involved in anencephaly.

We may assume, however, that the spontaneously occurring neural-tube defects are etiologically heterogeneous, with some factors causing only spina bifida or anencephaly and others causing both. Thorough pathologic comparisons between valproate-induced and "spontaneously" occurring defects might help define the extent to which the valproate-induced defects are phenocopies and have common pathogenetic backgrounds. The specific association also might result from metabolic or pharmacodynamic differences between the two target tissues - the rostral and caudal embryonic neural tissues.

The mean daily dose used by mothers with fetuses or babies with spina bifida was much higher than that used by mothers within the Rotterdam prenatal cohort who delivered normal babies (19). Dose-dependent and maternal serum level-dependent induction of neural-tube defects also was observed in experimental studies with pregnant rodents (20, 21) These findings suggest that the risk of spina bifida might be diminished by lowering the total daily dose, as well as by lowering the single doses by dividing the total daily dose into at least three doses per day.

The numbers for carbamazepine-exposed cases are too small to conclude that carbamazepine also is causally associated only with spina bifida and not with anencephaly.

All open defects but one were in the lumbosacral region of the spine, and often they were complicated by signs of hydrocephaly. There were three cases of closed spinal defects; in two of these, ascertainment might have been promoted through the presence of prominent symptoms: one case of sacrococcygeal teratoma and one case of bilateral radial aplasia, thumb hypoplasia, syndactyly, and polydactyly, in addition to multiple thoracic hemivertebrae and rib malformations. The third case was in a neonate with three unfused spinal arches detected during neonatal physical examination and confirmed radiographically.

The low proportion of closed spinal defects might be due in part to underdiagnosis and underreporting of this less dramatic type of congenital malformation. Only prospective studies with complete follow-up, including physical and ultrasonographic or radiographic examination, which control for parental findings and family history, might clarify whether valproate induces spina bifida aperta only or also induces closed spinal defects.

Association of birth defects with maternal antiepileptic drug use might reflect a causal association with the maternal disease itself, by inheritance or by transplacental mechanisms. The association between valproate exposure and spina bifida, however, is likely a causal one in the great majority of cases. The risk of spina bifida is much higher with valproate than with other currently used antiepileptic drugs (22), or as compared to historical controls before the introduction of valproate and carbamazepine. Furthermore, a specific genetic link between maternal epilepsy and fetal spina bifida - both are disorders of the central nervous system - is not found in the present study.

We observed no association of spina bifida with a specific type of maternal epilepsy. One-third of spinal defects in valproate-exposed offspring were associated with partial epilepsy, for which an association with genetic factors is less clear than for other types of epilepsy. The positive family histories of epilepsy or neural-tube defects were

equally distributed between the maternal and the paternal sides of the families and were not related to a specific type of maternal epilepsy (tables 2.3.3 to 2.3.5). This comparison between maternal and paternal families is particularly powerful, since there should be no major difference in recall between maternal and paternal relatives of the affected infant or fetus. We may assume that both sides of the family are equally involved in the drama of the birth of a handicapped relative and are equally aware of the nature of both the maternal and the neonatal condition.

Pharmacogenetic factors, on the contrary, might play a significant role in antiepileptic drug-induced teratogenesis. Four cases consisted of two pairs of siblings whose mothers were exposed to valproate during consecutive pregnancies. Two affected offspring were valproate-exposed monozygotic twins concordant for the defect. Previously, Robert *et al.* (23) also had reported a pair of monozygotic twins concordant for maternal valproate exposure and spina bifida aperta. To our knowledge, no cases of valproate exposure in monozygotic twin pairs discordant for spina bifida have been described so far. The observation of two pairs of valproate-exposed monozygotic twins concordant for spina bifida aperta is at least unexpected, in view of the low concordancy rate among monozygotic twin pairs not exposed to valproate (24). Studies of larger numbers of equally exposed siblings, dizygotic twins and monozygotic twins are necessary to evaluate the real contribution of predisposing genetic factors.

We also observe nine cases of spina bifida associated with maternal carbamazepine use. Six mothers were receiving carbamazepine monotherapy. We did not observe spina bifida to be associated with maternal use of any antiepileptic drugs other than valproate or carbamazepine. Phenobarbital, primidone, and to a lesser extent, phenytoin has been used less and less during the past 10 years, in favour of monotherapy. Monotherapy with valproate or carbamazepine has been used more frequently. This cannot explain the complete lack of primidone, phenobarbital, or phenytoin spina bifida cases in the present study, however. We used many different sources that each or each or together covered the entire period of 1972 through 1991.

Since 1984, a high proportion of affected valproate-exposed infants or fetuses has been accurately diagnosed prenatally. This shows that the information provided to the professional and lay public was effective, at least with regard to active referral for prenatal analysis. It also demonstrates that prenatal diagnosis of spina bifida aperta induced by valproate is possible and not essentially different from the diagnosis of spontaneously occurring open defects. We are not aware of false-negative results of prenatal analysis by the combined use of amniocentesis and structural ultrasonography. From 1984 on, valproate-exposed babies born with spina bifida were offspring of mothers who did not choose to terminate pregnancy after prenatal diagnosis. In two of these, the defect was found during ultrasound examination performed for obstetric reasons in the third trimester of pregnancy. We are aware of only one false-negative prenatal diagnosis in a carbamazepine-exposed case that was monitored only by fetal ultrasonography in mid-second trimester.

In the six-and-a-half year period from 1984 to 1990, the number of valproate-exposed spina bifida increased to 14, an average of two per year, as opposed to only 13

cases in the preceding period from 1972 to 1984, approximately one per year. This can be explained largely by use of valproate by women of childbearing age. Valproate use has increased to up to 30% of all patients being treated with antiepileptic drugs during the last 5 years (25). A change in reporting, and therefore ascertainment, can not be excluded. The Rotterdam prenatal cohort study started in 1985 and was conducted by a clinical genetics department that serves a regional population with 40,000 births per year. We identified cases that otherwise might have escaped detection. The Groningen EUROCAT registry started in 1981 and reached its present population coverage of approximately 19,000 births per year only recently, in 1989. On the other hand, after 1984, spina bifida teams contributed only a single case report, since the questionnaire sent out to these teams in 1984 was not sent out again. One or more additional midline defects within or outside the central nervous system were found in a considerable proportion of cases. This suggests that these different midline defects might share a common pathogenesis when observed in the general population, as well as in the valproate-exposed infants. Elucidating the mechanisms of valproate-induced teratogenesis might provide clues to the causes of neural-tube defects in the general population.

The association of valproate with bilateral radial aplasia in one of our cases, previously reported by Lindhout in 1985 (26), is probably not a chance finding, in view of subsequent reports in the literature (17, 27, 28), and the rarity of this type of defect in the general population. A similar type of skeletal defect is observed in rhesus monkeys prenatally exposed to high doses of valproate (29). Therefore, we recommend close monitoring of the fetal extremities during structural ultrasonography in pregnant women using valproate (30).

Spina bifida in infants exposed in utero to valproate was associated with significantly higher daily doses of valproate (>500 mg, or 50%) as compared to pregnancies in the Rotterdam prenatal cohort with normal outcome (19). Dose dependency of valproate teratogenicity has so far been observed only in animal experiments. The present findings suggest that reduction in daily doses, whenever possible, could lower the risk of spina bifida aperta.

A great difference was observed between the birth weights of full-term infants exposed to valproate only and those exposed to valproate in combination with other drugs. Previous experimental studies and human epidemiologic studies provided some evidence that comedication reduces the risk of valproate-induced neural-tube defects (9, 31) The pathogenetic mechanisms responsible for the lower birth weights of infants exposed to valproate polytherapy is unlikely to be the same as that responsible for spina bifida.

We conclude that valproate-induced neural-tube defects are usually severe, open spinal defects frequently complicated by hydrocephaly and other midline defects within or outside the central nervous system. Such birth defects can be diagnosed prenatally before the 20th week of pregnancy. Systematic follow-up of all valproate-induced cases of spina bifida should include post mortem examination. This complete follow-up might further delineate the spectrum of valproate-induced anomalies and shed light on the background of neurologic and behavioral abnormalities in valproate-exposed offspring without spinal defects. Primary prevention is possible by replacing valproate with another antiepileptic

drug before pregnancy, but this policy is complicated by the fact that an absolutely teratogen-free antiepileptic drug has yet to be discovered. High daily doses of valproate, *i.e.* > 1,000 mg, probably have a higher risk than do lower doses. When the use of valproate cannot be avoided, a reduction in the total daily dose, if possible, and the division the daily dose into three or more equal doses administered throughout the day, seem to be wise measures. The spectrum of carbamazepine-associated neural-tube defects and other birth-associated malformations is not yet clear.

2.3.5 References

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PRENATAL DIAGNOSIS OF SPINA BIFIDA APERTA AFTER FIRST-TRIMESTER VALPROATE EXPOSURE

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Abstract

In the context of a prospective study on the adverse effects of anti-epileptic drugs on fetal outcome, we evaluated our experience with prenatal diagnosis by ultrasonography and α-fetoprotein (AFP) determination in amniotic fluid. We compared these results with AFP values in maternal serum obtained prior to amniocentesis/ From November 1985 to July 1990, amniocentesis at 16-18 weeks of gestation was performed in 267 pregnancies of 237 different women using antiepileptic drugs. Among 92 pregnancies with maternal valproic acid use, five (including one concordantly affected monozygotic pair of twins) were terminated because of a spina bifida aperta, all prenatally diagnosed by AFP determination and acetylcholinesterase electrophoresis in amniotic fluid. The maternal serum AFP level was raised (≥2.5 multiples of the median (MOM) for singleton pregnancies and ≥4.5 MOM for twin pregnancies) in only two of these five affected pregnancies. We emphasize that maternal serum AFP levels may be unreliable for prenatal screening for fetal neural tube defects in women taking valproate and recommend that amniocentesis and fetal ultrasound examination should be offered directly.

2.4.1 Introduction

Elevated levels of α-fetoprotein in maternal serum will, after exclusion of other causes, identify a group at increased risk for specific congenital malformations, such as neural tube defects. Elevated maternal serum α-fetoprotein levels are by no means specific for neural tube defects, but may also be due to, for example, underestimated gestational age, multiple gestation, or fetal death. In the "standard" protocol used in several countries with population-based screening programmes (UK, USA, Cuba, etc) (1) elevated maternal serum α-fetoprotein levels (on two occasions) is an indication for ultrasonography, which often reveals an explanation for the elevated levels. If no obvious reason is found, amniocentesis is recommended in which amniotic fluid α-fetoprotein is determinated, and in case of an equivocal α-fetoprotein level, acetylcholinesterase electrophoresis is carried out.

In The Netherlands, no population-based maternal serum α-fetoprotein screening program is operative. Maternal serum α-fetoprotein determination is provided in a few hospitals only, with the serum analysis performed at the National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands. In our clinic, amniocentesis is offered directly to women known to be at increased risk, based on a positive family history or other known risk factors such as maternal diabetes mellitus and antiepileptic drug use.

The use of the antiepileptic drug valproic acid during the first trimester of pregnancy is associated with a 1-2% risk of neural tube defects (2, 3). For carbamazepine the risk of neural tube defects is estimated at 1% (4, 5, 6). Since 1984 (4), maternal use of these drugs has been added to the list of official indications for amniocentesis and α-fetoprotein determination. Since the estimated risk of the more classic antiepileptic drugs (*e.g.* phenobarbital, primidone and phenytoin) is estimated to be 0.3-0.4% (7), which is a 2 to 3 fold risk compared to the general Dutch population, ultrasound is offered to these women and an amniocentesis is performed only on the patient's request. In the context of a prospective study on the adverse effects of antiepileptic drugs on fetal outcome, we evaluated our experience with prenatal diagnosis by ultrasonography and α-fetoprotein (confirmed by acetylcholinesterase) and compared these data with α-fetoprotein values in maternal serum, obtained just prior to amniocentesis.

2.4.2 Methods

The protocol was approved by the Medical Ethics Committee of the Erasmus University Rotterdam and the University Hospital Rotterdam Dijkzigt. The population studied consisted of 297 pregnant epileptic patients using antiepileptic drugs. They were referred for prenatal diagnosis of neural tube defects or other major malformations by amniocentesis at 16-18 weeks of gestation and/or detailed ultrasound examination at 18-22 weeks of gestation. In 267 pregnancies (of 237 different women) amniocentesis was performed, in 201 of which maternal serum was also obtained immediately prior to

amniocentesis. Amniotic fluid was tested for α-fetoprotein levels by radial immunodiffusion (8). In the case of elevated α-fetoprotein levels, the diagnosis of neural tube defects was confirmed by amniotic fluid acetylcholinesterase banding by gel-electrophoresis (9) and repeated ultrasonography. Maternal serum α-fetoprotein was tested at the National Institute of Public Health and Environmental Protection (RIVM), Bilthoven with an "in house" ELISA method (10) and considered elevated when values were above 2.5 multiples of the median (MOM) for singleton pregnancies and 4.5 MOM for twin pregnancies. Analysis of variance was performed after transformation of the MOM to the base 10 logarithm. After termination of pregnancy, all fetuses were examined by visual inspection. Autopsy was performed on only four of the fetuses, since no permission was obtained for the pair of twins.

2.4.3 Results

In the group under study as a whole (pregnant women using antiepileptic drugs), the geometric mean of the multiples of the medians of maternal serum α-fetoprotein did not differ significantly from the reference values based on more than 24.000 determinations over the period of 1977-1990 in the institute mentioned above. Analysis of variance revealed no differences in the MOMs of maternal serum α-fetoprotein between the different medications as mono- or poly-therapy.

In this study, six fetuses with an open spina bifida were diagnosed prenatally (table 2.4.1). Two of them were a pair of twins and turned out to be monozygotic and concordant for spina bifida. In all five pregnancies valproate was used, in one case in combination with carbamazepine (11). In five of the 201 pregnancies maternal serum α-fetoprotein was elevated. Two out of these five were due to an open neural tube defect (table 2.4.1). No obvious reasons could be found for the remaining three during follow-up until birth. In this cohort, two fetuses died within 1 week after amniocentesis, one due to an intrauterine infection and one intrauterine fetal demise in a twin pregnancy.

Table 2.4.1 Prenatal diagnosis of spina bifida after first trimester valproic acid exposure.

Case	Gestational age diagnosis (weeks)	TOP	First diagnosis US	AF-AFP ($\mu g.ml^{-1}$)	Maternal serum AFP ($\mu g.ml^{-1}$)	MOM	Amniotic fluid AFP	(limit)	AchE	Final diagnosis and karyotype
1	16	17	-	+	141	3.8	127	(40)	+	MMC (& H), 46,XY
2a	18.5	20	+	-	111	3.1	60	(12)	+	MMC (& H), 46,XY
2b	18.5	20	-	+			69	(12)	+	MMC (& H), 46,XY
3	15.5	16	+	-	21	1.0	42	(38)	+	MMC (& H), 46,XX
4	16	17	-	+	30	1.2	61	(40)	+	MMC , 46,XY
5	18	20	-	+	94	2.6	33	(25)	+	MC (& H), 46,XX

TOP = termination of pregnancy; US = ultrasonographic examination, AF-AFP = α-fetoprotein ($\mu g.ml^{-1}$) determination in amniotic fluid, MOM = multiples of the median, cut-off level for singleton pregnancies 2.5 MOM and for twin pregnancies 4.5 MOM. AchE = acetylcholinesterase banding, MMC = myelomeningocele, MC = meningocele, H = hydrocephalus, based on dilated ventricles observed with ultrasonography.

2.4.4 Discussion

In only two of five pregnancies with a fetal neural tube defect was maternal serum α-fetoprotein higher than the cut-off level of 2.5 MOM for singleton pregnancies or 4.5 MOM for twin pregnancies. In the remaining three affected pregnancies, maternal serum α-fetoprotein levels did not exceed the cut-off level and these women would not have benefited from the diagnostic work-up of pregnancies had we relied upon maternal serum α-fetoprotein levels alone.

Whereas normally 70-90% of the neural tube defects are detected by maternal serum α-fetoprotein screening (unpublished report RIVM, 12), in this cohort only 40% (2/5) were properly diagnosed this way. Since the total group of women receiving valproate did not have maternal serum α-fetoprotein levels different from those found in the general population or from women on other antiepileptic drugs, we presume that the lower maternal serum α-fetoprotein levels may have more to do with the type and degree of neural tube defects than with valproate medication itself.

With case 5, the importance of an accurate determination of the gestational age is demonstrated once more, also when α-fetoprotein analysis in amniotic fluid is applied. In this case the α-fetoprotein level was 33 $\mu g.ml^{-1}$ with an uncertain gestational age of 17 weeks. With an upper limit for α-fetoprotein in amniotic fluid at 17 weeks of 35 $\mu g.ml^{-1}$, this value is borderline. Re-evaluation of the proper gestational age showed a more advanced gestation (18 weeks, α-fetoprotein upper limit 25 $\mu g.ml^{-1}$). Spina bifida may be associated with a smaller biparietal diameter at ultrasound examination (13). If not recognized as such, this may lead to an unintentional underestimation of the gestational age and misinterpretion of α-fetoprotein levels. Five of the six fetuses showed lemon sign or dilated ventricles on ultrasound examination, which might be indicative for an associated hydrocephaly (13). These hydrocephalies could not be confirmed at autopsy, except for one case, due to extensive fetal maceration.

In two cases, the fetal neural tube defect was first seen on ultrasound and confirmed by raised α-fetoprotein levels in amniotic fluid. In the other four cases, ultrasound confirmation was obtained after finding abnormal α-fetoprotein concentrations and positive acetylcholinesterase banding in amniotic fluid. Although some authors (14) recommend high resolution ultrasound rather than amniocentesis to women of a high risk population, this is not the practice in The Netherlands (15). Maternal serum α-fetoprotein measurement is a screening test and therefore not appropriate for a high-risk population, as we have shown here for women using valproate, and we recommend that all women at increased risk of fetal neural tube defects due to valproate medication should be offered prenatal diagnosis by amniocentesis with α-fetoprotein analysis and fetal ultrasound examination.

2.4.5 References

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RISK FACTORS FOR MAJOR CONGENITAL ANOMALIES IN THE OFFSPRING OF TREATED EPILEPTIC WOMEN

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Abstract

This paper describes the findings of a prospective study of 297 pregnancies of 261 different epileptic women treated with antiepileptic drugs, who were referred to our clinic for prenatal diagnosis. The women entered the study before 22 weeks of gestation and follow-up was up to three months after birth. In 76% of the pregnancies monotherapy was used. Carbamazepine was the most frequently used drug (54%), either as monotherapy (38%) or polytherapy (16). 6.7% (20/297) of the pregnancies ended with malformed offspring; 6.2% (15/226) with monotherapy and 8.5% (6/71) with polytherapy. Besides spina bifida (n=5, in association with valproic acid), congenital heart defects (n=2, with phenobarbital), hypospadias (n=2, with carbamazepine), facial clefts (n=3), a variety of other anomalies was observed. Possible factors contributing to an increased risk for congenital anomalies other than spina bifida included partial epilepsy and a positive family history of epilepsy also on the paternal side. Relative risk estimates, adjusted for these factors, were slightly lower for phenytoin or carbamazepine than for phenobarbital or valproate.

2.5.1 Introduction

The literature on the possible teratogenic effects of antiepileptic drugs is abundant. The first study on this subject appeared in 1964 (1). Trimethadione became known as one of the most potent human teratogens and as such contraindicated during pregnancy (2). Other antiepileptic drugs, like phenytoin and phenobarbital, which are more commonly used during the reproductive age, have also been associated with increased malformation rates. With these drugs, especially the prevalence of cleft lip with or without cleft palate and congenital heart defects seems to be increased (3). In general, maternal epilepsy and the use of anticonvulsants is associated with a two to three fold increase in congenital anomalies in the offspring (3). With more data becoming available, the possible role of a genetic susceptibility whether or not associated with the epilepsy or the treatment, and the contribution of the underlying epilepsy to the observed increased malformation risk became a matter of discussion (3).

Partly because of warnings about the possible teratogenic potential, especially when more than one anticonvulsant was used (4, 5), and partly because of the introduction of new drugs, prescription policies have changed over the years. About 15 years after its introduction in Europe, valproate was found to be associated with a risk of 1-2% of spina bifida (6, 7). Soon thereafter, this was also suggested for carbamazepine (7, 8). For valproate the causality of this association was soon generally accepted (9), but with respect to carbamazepine it took almost 10 years to accumulate enough data to estimate the risk at approximately 0.9% (10). This makes the choice of therapy more difficult, since carbamazepine has long been considered as the safest alternative for use in women of childbearing age. Continuation of therapy during pregnancy is a necessity for most epileptic women. The clinically important question which antiepileptic drug has the lowest teratogenic risk in a particular patient can still not be answered satisfactory.

This paper describes our experience over a period of almost 5 years in a prenatal cohort of treated maternal epilepsy in which carbamazepine was the drug most frequently used, either as monotherapy or polytherapy. Besides the medication, attention is given to the role of other factors, like the epileptic condition of the mother and the family histories on birth defects and epilepsy. Since the association between valproate and spina bifida was already well established in an earlier analysis of this cohort (11), this paper focuses especially on non-spinal defects and their risk factors.

2.5.2 Patients and methods

The protocol of the study was approved by the Medical Ethics Committee of the Erasmus University Rotterdam and the University Hospital Rotterdam Dijkzigt. The study population consisted of pregnant epileptic women, using antiepileptic drugs. Subjects were referred to the outpatient clinic for prenatal diagnosis of neural tube defects and other major malformations by amniocentesis at 16-18 weeks of gestation and/or structural ultrasound examination at 18-22 weeks of gestation. After informed consent was obtained

a standardized questionnaire was filled in comprising questions on maternal age, length, weight before pregnancy and at intake, paternal age, duration of epilepsy, seizure frequency before and during the first trimester of pregnancy, occurrence of seizures with cyanosis, drug regimen (formulation, dose and dosing schedule), obstetric history, complications during pregnancy and other chronic or intercurrent maternal illnesses, medication or exposures. A standardized family history was taken concerning the presence or absence of epilepsy, chronic or hereditary diseases, malformations and mental retardation in first and second degree relatives of the parents as well as in more remote relatives. The participants were assured that the medication should be continued as prescribed by the neurologist and were asked to report at amniocentesis whether there had been any change in medication during the interval between intake and amniocentesis.

In the analysis of dose-outcome relationship the dosages used during the first eight weeks of pregnancy were taken. More precise information about the underlying epilepsy and obstetric history, complications and outcome of this pregnancy was obtained through the woman's neurologist and obstetrician. Epilepsies and seizures were classified according to the International Classification of Epilepsies, Epileptic Syndromes and Related Seizure Disorders of the International League Against Epilepsy (12). The seizure free period before pregnancy and the occurrence of generalized seizures during pregnancy were used as indicators for the severity of the underlying epilepsy.

Gestational age was calculated from the first day of the last menstrual period and from the ultrasonic measurements of crown-rump length or biparietal diameter. Infants with birth measurements below the 3rd centile (13) were categorized as small for gestational age or in case of head circumference as microcephalic. Only major congenital abnormalities or malformations, i.e. requiring treatment, were included and only if the diagnosis was made within the first 3 months of life, or suspected and confirmed by diagnostic procedures initiated within this period. Excluded from this analysis were structural balanced chromosomal abnormalities for which one of the parents was a carrier ($n=1$), as well as dominant diseases inherited from one of the parents ($n=1$). After delivery, an inquiry was made about any significant changes in medication during the 2nd and 3rd trimester, the occurrence of generalized tonic-clonic seizures or status epilepticus, the mode of delivery and the infant's welfare.

For proportions 95% confidence limits were calculated. Chi-square statistics were used for the univariate analysis of categorical data. Crude and adjusted relative risks for the occurrence of malformations were calculated for each antiepileptic drug, used alone or in combination with others, against the use of any other antiepileptic drug exposure. Logistic regression analysis was applied to estimate the adjusted relative risks of congenital abnormalities associated with the different antiepileptic drugs and other possible risk factors. Since the spinal defects were all associated with valproate and not with any other relevant factor (11), a distinction was made between spina bifida cases and other congenital abnormalities, further referred to as non-spinal defects, in these analyses.

Table 2.5.1 Drug use during the first trimester of pregnancy in a cohort of 297 pregnancies

1 drug regimen			2 drugs regimens			≥ 3 drugs regimens		
CBZ	114	(50%)	CBZ & VPA	13		CBZ & VPA & DPH	1	
VPA	60	(27%)	CBZ & DPH	6		CBZ & VPA & ESX	2	
DPH	28	(12%)	CBZ & PHB	9		CBZ & VPA & CLP	1	
PHB	18	(8%)	CBZ & CLP	7		CBZ & VPA & CLM	1	
PMD	-		CBZ & CLM	2		CBZ & VPA & DZP	1	
ESX	2		VPA & DPH	2		CBZ & PMD & CLM	1	
DZP	1		VPA & PHB	5		CBZ & PMD & CLM	1	
CLM	2		VPA & ESX	1		CBZ & PHB & DPH	1	
CLP	-		VPA & CLP	3		VPA & PHB & PMD	1	
ACA	1		VPA & CLZ	1		VPA & PHB & ESX	1	
			DPH & PHB	7				
			DPH & CLP	1		CBZ & DPH & PHB & CLM	1	
			PHB & PMD	1				
			ESX & CLP	1				
Total	226	(76%)		59	(20%)		12	(4%)

CBZ = carbamazepine; VPA = valproate; DPH = phenytoin; PHB = phenobarbital; PMD = primidone; ESX = ethosuximide; DZP = diazepam; CLM = clobazam; CLP = clonazepam; ACA = acetozolamide

2.5.3 Results

From November 1, 1985, until July 1, 1990 291 singleton and 6 twin pregnancies entered the study, resulting in 303 fetuses. Table 2.5.1 shows the precise drugs used, alone or in combination, during the first trimester of pregnancy. In 76% of pregnancies monotherapy was used, of which in 50% carbamazepine and in 27% valproate.

Two spontaneous abortions before 24 weeks of gestation, 1 stillbirth after placental abruption and 2 neonatal deaths due to respiratory failure of prematurely delivered infants occurred. In 21 infants (or fetuses) a major anomaly was encountered (table 2.5.2), including one pair of monozygotic twins, concordantly affected (*i.e.* 20 pregnancies with abnormal outcome). The difference in the occurrence of major malformations observed between monotherapy (14/226, 6.2%) and polytherapy (6/71, 8.5%) did not reach significance. The relative risk estimate for polytherapy compared to monotherapy was 1.4 (95% CI, 0.5 to 3.4). No malformations were seen with phenytoin monotherapy (0/28) and only a low frequency was seen with carbamazepine monotherapy (3.5%). Crude relative risk estimates for the occurrence of all types of malformations (*i.e.* spinal and non-spinal) for each specific drug relative to all other exposures, were for carbamazepine 0.7 (95% CI, 0.3 to 1.7), valproate 1.5 (95% CI, 0.6 to 3.5), phenytoin 0.3 (95% CI, 0.04 to 2.0) and phenobarbital 1.4 (95% CI, 0.5 to 4.1), respectively (fig. 2.5.1).

Whereas for spinal defects it could be demonstrated that significantly higher daily valproate doses were used (M $\pm$ SEM: 1640 $\pm$ 136 $mg.d^{-1}$ vs. 941 $\pm$ 48 $mg.d^{-1}$,

Table 2.5.2 Malformed offspring, anticonvulsant or other drugs exposure and other risk factors

Nr.	Type of congenital anomalies	DPH	PHB	PMD	CBZ	VPA	CLP	CLM	ACA	Other drugs	Type of epilepsy	Previous miscarriages or stillbirth	Family history of epilepsy mat/pat
1.♂	Spina bifida aperta	-	-	-	-	1700	-	-	-		PG	-	-/-
2.♂	*Spina bifida aperta	-	-	-	-	1800	-	-	-		PG	-	-/-
3.♂	*Spina bifida aperta												
4.♂	Spina bifida aperta	-	-	-	-	1200	-	-	-		PG	-	-/-
5.♀	Spina bifida aperta	-	-	-	-	2000	-	-	-		P	sp.ab.	+/+
6.♀	Spina bifida aperta	-	-	-	600	1500	-	-	-		P	-	-/-
7.♂	Hypospadia, hydrocephalus, cleft lip and palate	-	-	-	200	-	-	-	-		PG	-	-/-
8.♂	Cleft lip	-	150	-	-	-	-	-	-		P	-	-/-
9.♀	Cleft palate (soft total, hard 1/3)	-	-	-	800	1200	-	-	-	salbutamol, beclomethason	?	-	-/-
10.♀	Congenital heart defects (VSD, ASD, PS, ODB)	-	150	-	-	-	-	-	-		P	st.b.	-/-
11.♀	Club foot left	-	-	-	600	-	-	-	-		PG	-	-/-
12.♂	Club foot right	-	-	-	-	1000	-	-	-		P	-	-/-
13.♀	Microcephaly (<-3SD)	-	-	750	600	-	-	30	-		P	-	+/+
14.♂	Inguinal hernia	-	-	-	-	750	-	-	-		U	-	+/-
15.♀	VSD, SASDS, radiusaplasia left hemivertebrae (1,3 & 5 chest)	-	150	-	-	-	-	-	-		P	-	+/-
16.♂	Hypospadia, ptosis (S.Horner)	-	-	-	600	-	-	-	-	temazepam	P	-	-/-
17.♂	Hypertrophic pylorusstenosis	-	-	-	400	-	-	-	-		P	-	-/-
18.♀	PMR, seizures, strabismus divergens	300	30	-	-	-	-	-	-	alcohol abuse	P	6 sp.ab., 1 st.b.	+/?
19.♂	†IgG-deficiency, epilepsy, heart murmur	-	400	-	2	-	-				P	sp.ab.	+/+
20.♂	†IgA-deficiency, thymushypoplasia	-	-	-	400	-	2	-	-	phenprocoumon	"	"	"
21.♀	Chylothorax & persistent fetal circulation	-	-	-	-	-	-	-	250	meclozine, pyridoxine	P	sp.ab.	-/+

VSD = ventricular septal defect, ASD = atrial septal defect; ODB = open ductus botalli; PS = pulmonary artery stenosis; SASDS = subclavian artery supply dysruption sequence, PMR = psychomotor retardation, mat. = maternal, pat. = paternal.

* Twin-twin transfusion, case 2 donor, case 3 acceptor; † sibs, no known immune deficiency syndrome; parents immune competent and not consanguineous, and family history was negative for immune deficiency.

p=0.0001) (11), such differences were not found for carbamazepine, valproate, phenytoin and phenobarbital when non-spinal defects were analyzed. Mothers with malformed offspring used besides antiepileptic drugs more additional medication (table 2.5.2).

Since spina bifida appears to be causally related to valproate (11) we excluded these spina bifida cases from the subsequent analysis, which focuses on the question as to whether the other observed defects (further referred to as non-spinal defects) are related to a particular treatment or more to other risk factors.

There were no clear differences between the women with malformed and normal offspring with respect to maternal age, weight, social class and marital status (table 2.5.3 and 2.5.4). Comparison of the obstetric histories between both groups revealed no significant differences in number of induced or spontaneous abortions, previous premature deliveries or neonatal deaths. 47% of the mothers with malformed offspring were primigravidae. Of the multigravidae, 2 women with offspring with non-spinal defects (n=15) had experienced a previous stillbirth versus 4 women in the group with normal offspring (n=277) (table 2.5.4).

Table 2.5.3 Maternal characteristics according to outcome of pregnancy

	non-spinal defects n=15		normal n=277		p-value
	M ± SEM	(range)	M ± SEM	(range)	
Maternal age (yrs)	28±1	(20-38)	29±0.4	(17-41)	0.63
Height (cm)	164±2	(152-182)	168±0.4	(147-189)	0.07
Weight before pregnancy (kg)	63±3	(49-86)	65±1.0	(37-133)	0.47

The only significant difference between both groups with regard to family history was concerned with previous malformed offspring. This significance could be attributed to the two affected siblings (case nos. 19 and 20). In this family a previous child was born with an atrium-septum defect and an IgA deficiency; also in this pregnancy carbamazepine was used. If only the first pregnancy of the woman in the study was included, the difference for previous malformed offspring was not significant any more (p=0.35). Comparison of the number of positive family histories of epilepsy or mental retardation on the maternal side (non-spinal defects vs. normal outcome) revealed no significant differences. However, more malformed infants had a paternal family history (with exclusion of the father himself) of epilepsy as well as mental retardation (table 2.5.4).

With regard to maternal epilepsy characteristics, none of the parameters studied showed statistically significant differences (table 2.5.4). However, slightly more mothers of malformed offspring had partial epilepsy and experienced focal seizures with secondary generalization. Likewise, generalized epileptic seizures occurred in 20% of pregnancies in the first trimester and this percentage was slightly, but not significantly higher for malformed outcome compared to normal pregnancy outcome.

Table 2.5.4 Maternal (and paternal) characteristics according to outcome of pregnancy

	non-spinal defects n=15		normal n=277		p-value
	nr.	(%)	nr.	(%)	
Married status (yes)	13	(87)	233	(84)	0.79
Social class					0.71
academic degree	0	(0)	16	(6)	
non-ac. white collar	7	(47)	93	(34)	
skilled manual labor	4	(27)	8	(28)	
unskilled manual labor	2	(13)	33	(12)	
unknown	2	(13)	57	(21)	
Consanguinity (yes)	0	(0)	5	(2)	-
OC failure	0	(0)	16	(6)	
Primigravidae	7	(47)	109	(39)	0.58
Previous miscarriage or stillbirth	5	(33)	54	(20)	0.22
Previous malf.offspring	3	(20)	14	(5)	0.05
Maternal malformations	3	(20)	18	(7)	0.10
Maternal family history (≥2nd degree) positive on epilepsy	4	(27)	66	(24)	0.14
Paternal epilepsy	0	(0)	4	(1)	
Paternal malformations	0	(0)	15	(5)	0.20
Paternal family history (≥2nd degree) positive on epilepsy	4	(27)	20	(7)	0.02
Type of epilepsy				0.05	
primary generalyzed	2	(13)	116	(42)	
partial	11	(73)	129	(47)	
unclassifiable/unknown	2	(13)	32	(12)	
Early onset of epilepsy (<20 yrs)	13	(87)	201	(73)	0.34
Organic basis of epilepsy†	1	(5)	50	(18)	0.21
Duration of epilepsy (>10 yrs)	8	(53)	191	(69)	0.22
More than 5 yrs seizure free	1	(7)	36	(13)	0.16
Generalized seizures	13	(87)	212	(77)	0.34
Experiencing generalized seizures					
- during pregnancy any time	7	(54)	65	(31)	0.11
- during first trimester	4	(31)	40	(19)	0.34

* One woman counts twice, i.e. the parents of both affected sibs had a previous malformed child, † organic basis of epilepsy might be perinatal, posttraumatic or postencephalitic.

Estimates of relative risks were obtained using logistic regression analysis, taking into account these factors which appeared to be relevant after univariate analysis; i.e. type of epilepsy (partial epilepsy increased the risk), previous miscarriages or stillbirth (increased risk) and a positive family history for epilepsy (increased risk) (table 2.5.4). For the separate antiepileptic drugs relative risk estimates were adjusted for these factors (fig. 2.5.1). Although the absolute figures are slightly different from the crude relative risk estimates, the order of risks remains the same. No term for exposure for any of the drugs relative to other exposures, on malformations other than spina bifida became

significant. Although phenobarbital and valproate appear associated with a slightly higher risk for congenital anomalies than carbamazepine and phenytoin, the differences are not statistically significant.

Table 2.5.5 Relative risk estimates for some risk factors adjusted for different antiepileptic drugs used

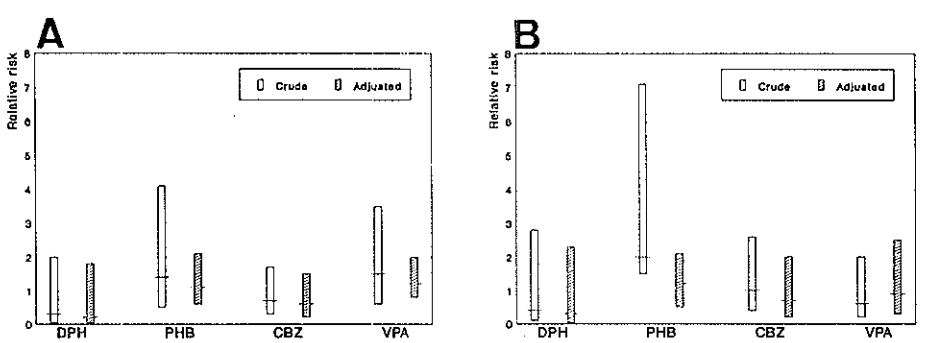
	Non-spinal defects, only Relative risk (95% CI)		All defects Relative risk (95% CL)	
Type of epilepsy *				
primary generalized	0.3	(0.1 - 1.1)	0.5	(0.2 - 1.1)
partial	2.3	(0.9 - 5.5)	1.9	(0.9 - 5.5)
Previous miscarriages (no/yes)	1.2	(0.3 - 4.5)	1.1	(0.3 - 4.5)
Family history on epilepsy (neg/pos)				
positive on maternal side	1.7	(0.5 - 1.8)	1.0	(0.3 - 3.1)
positive on paternal side	3.6	(0.8 -17.0)	3.9	(1.0 - 15.0)

* Risk relative to all other categories, including unclassifiable or unknown epilepsy types.

2.5.4 Discussion

When all anomalies are included the relative risk estimate for polytherapy compared to monotherapy is 1.4 (95% CI, 0.5 to 3.4). Several studies have shown that polytherapy is associated with a higher risk for birth defects than monotherapy (3, 4, 5). In those previous studies 70 to 80% of the patients were treated with polytherapy. Over

Fig. 2.5.1 Risk estimates and 95% CI for the occurrence of all type of defects (A), and for non-spinal defects seperately (B), for each of the four antiepileptic drugs, relative to all other exposures, both crude and adjusted for other risk factors, *i.e.* type of epilepsy, a positive family history for epilepsy and previous miscarriages or stillbirths



DPH = phenytoin, PHB = phenobarbital, CBZ = carbamazepine, VPA = valproic acid

the years a shift towards monotherapy has occurred and meanwhile in this study, the situation is reversed, with 76% of the women treated with monotherapy. This allows for a better evaluation of the risks of single antiepileptic drugs.

Although prospective studies have the advantage of avoiding recall bias problems and provide denominator data for exposures, they often have limitations with respect to size. Similarly in this study, the small number of malformed infants limits the statistical power. Furthermore, anomalies encountered in cases 18 to 21 are difficult to interpret, because these are not structural defects with a known window of sensitivity. All four infants were born prematurely and one could argue whether these abnormalities should be included or not. Therefore, we have provided the detailed tables 2.5.1 and 2.5.2, which allows any desired recalculation of malformation rates by the reader.

The analysis on possible risk factors for the occurrence of non-spinal defects in infants of women on antiepileptic medication revealed that especially with a positive family history of epilepsy on the paternal side the risk was 3 to 4 times increased. Whereas none of the fathers of malformed offspring had epilepsy himself (against 4 in the group with normal outcome) more second degree and more remote family members were known with epilepsy, *i.e.* 27% vs. 7%. Given the multifactorial etiology of both epilepsy (14) and major congenital malformations (15) involving the interaction of multiple genes with environmental factors, this finding might reflect inheritance of certain liability genes, also from the father's side, for major congenital malformations which show familial aggregation with epilepsy.

Familial aggregation has been suggested based on increased (i.e. compared with the general population) epilepsy prevalences in relatives of patients with malformations (16-18), as well as increased malformation rates in relatives of epileptic patients (19, 20). Although data are conflicting in this regard, the most recent and elaborate studies demonstrate an increased risk only for the progeny of treated epileptic women (20, 21). Comparison of malformation rates between the progeny of epileptic fathers and the general population show variable results. Whereas some studies found slightly higher prevalence of major congenital malformations among the offspring of epileptic men (22-24), others did not find a prevalence rate exceeding that of the general population (25-26). The tendency of malformations to recur in families might also suggest a greater role for inheritance, than the potential teratogenic effects of antiepileptic drug treatment. However, if in both pregnancies the same medication is being used this might also be a sign of genetic predisposition to the teratogenic effects of these drugs.
In our study partial epilepsy is associated with a 2 times higher risk on malformed offspring than other forms of epilepsy. Also in this respect controversy exists in the available literature. Whereas in earlier studies a preponderance of the more genetically determined primary generalized epilepsies was reported (27), in more recent and larger studies congenital malformations occurred more frequently in the offspring of mothers suffering from partial epilepsy (4, 5).

No relationship was found with maternal age, opposite to what was suggested by other reports (3). Although more malformed infants were born from primipara mothers (60%), more previous stillbirths were observed in the multipara mothers with malformed

offspring. The relative risk of previous miscarriages or stillbirth adjusted for the two above mentioned risk factors as well as the medication used, however, turns out to be close to unity.

In this cohort hypospadias was encountered twice after carbamazepine monotherapy. Six cases of hypospadias after carbamazepine exposure have been reported, previously (28, 29, 30, 31), of which two in combination with valproate. A specific association between hypospadias and valproate has already been suggested (7, 31, 32). However, hypospadias is a rather common anomaly with a prevalence at birth of 0.1% to 0.2% and known to be an underreported anomaly in birth defect registries with an estimated ascertainment of 40-80% (31). The increasing number of prospective studies on the possible teratogenic effects of antiepileptic drugs over the years with a concomitant increase in the use of valproic acid and carbamazepine, might also be an explanation for the more frequently observed hypospadias with these two drugs. Therefore, it remains to be established whether carbamazepine and valproate are specifically associated with hypospadias.

In conclusion, after more than two decades of studying the occurrence of congenital anomalies in the offspring of epileptic patients it still remains extremely difficult to distinguish which factors are particularly important in increasing the risk of major malformations observed in treated epileptic mothers and to establish the drug with the lowest risk. Our data, as well as other studies, indicate that especially with partial epilepsy more malformed offspring is observed and furthermore, that the risk of malformed offspring increases when the family history is positive for epilepsy, especially on the paternal side. Although data are controversial whether there is a familial aggregation between epilepsy and a wide range of congenital anomalies, these results make it tempting to assume certain liability genes for congenital anomalies present in those families. In this cohort we found valproate and phenobarbital associated with slightly higher malformation risks. From the teratogenic point of view, monotherapy with carbamazepine is, if therapeutically suitable, may be a good alternative to treat epileptic women who are planning a pregnancy and need treatment.

2.5.5 References

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THE DISPOSITION OF VALPROATE AND ITS METABOLITES IN THE LATE FIRST TRIMESTER AND THE EARLY SECOND TRIMESTER OF PREGNANCY IN MATERNAL SERUM, URINE, AND AMNIOTIC FLUID: EFFECT OF DOSE, COMEDICATION AND THE PRESENCE OF SPINA BIFIDA

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Abstract

In 60 pregnancies in epileptic women taking long-term valproate we have measured the concentrations of the parent compound and 13 of its metabolites by gas-chromatography-mass-spectrometry in amniotic fluid, maternal serum, and 24 h maternal urine samples. All metabolites of valproate present in the serum could also be detected in the amniotic fluid, although at much lower concentrations. Amniotic fluid concentrations of valproate and several of its metabolites ((E)Δ^2-valproate, (2E,3'E)$\Delta^{2,3'}$-valproate and 3-keto-valproate) correlated with total valproate concentrations as well as with unbound valproate concentrations in maternal serum. We suggest that the amniotic fluid acts as a deep compartment, with slow appearance and disappearance of valproate and its main metabolites. The data further suggest that during the first and early second trimesters of pregnancy the ß-oxidation of valproate decrease. In pregnancies associated with fetal neural tube defects (n=5) significantly higher daily doses of valproate were used, compared with normal pregnancies (n=54). This resulted in higher concentrations of valproate in maternal serum.The metabolite patterns in maternal serum, 24 h urine samples, and amniotic fluid did not show any significant differences in pregnancies with neural tube defects.

2.6.1 Introduction

Valproate (2-n-propyl-pentanoic acid) is widely used in the treatment of idiopathic generalized epilepsy. Its long-term use in women of child-bearing age is hampered by the potential risk of teratogenicity and embryotoxicity (1, 2). The risk of neural tube defects is about 1-2%, a 10 to 20 fold increase compared with the prevalence at birth in most populations (2). Despite this relative contraindication valproate continues to be given to pregnant women, presumably because in individual patients the therapeutic benefits are considered to outweigh the fetal risks and pregnancy occurs while on prescription.

In comparison with other major antiepileptic drugs, little information is available concerning the disposition of valproate during pregnancy. Some data are available on the placental transfer of valproate at term (3-7), but few studies have focused on early pregnancy.

In the Netherlands prenatal diagnosis for open neural tube defects by amniocentesis is offered to women using valproate during the first trimester of pregnancy since 1984. (8). This provided us with the chance of studying the distribution of valproate in the amniotic fluid in the early second trimester.

2.6.2 Materials, methods and subjects studied

Study population. We studied pregnant epileptic patients referred to the outpatient clinic for prenatal diagnosis of the University Hospital Rotterdam Dijkzigt, because of the increased risk of neural tube defects associated with valproate. Some women had other indications for prenatal diagnosis as well, mainly the increased risk of a chromosomal abnormality connected with a maternal age of 36 or older. A more precise description of the clinical protocol and characteristics of these patients is published elsewhere (9).

At the first consultation (further referred to as intake, 6-14 weeks of gestation) the study was explained and a letter containing detailed information was given. When informed consent had been obtained data were collected on, among other things: maternal age, height, weight before pregnancy and at intake, and drug regimen. The protocol was approved by the Medical Ethics Committee of the Erasmus University Rotterdam and the University Hospital Rotterdam Dijkzigt.

Sample collection and storage. Blood samples were obtained, randomly within the dosing-interval. After allowing the blood to clot, the serum was separated, frozen, and stored at -80°C until analysis. The day before amniocentesis the patient collected her urine for 24 h and stored it in the refrigerator. At the day of amniocentesis, the volume of the urine was determined and a sample was stored at -80°C until analysis. Ten ml (or 20 ml if chromosome analysis was also required) of amniotic fluid was collected and after α-fetoprotein determination the remaining supernatant was stored at -80°C.

In a twin pregnancy, with both fetuses affected by lumbosacral spina bifida aperta, amniocentesis of both amniotic compartments was repeated before the termination of pregnancy, and fetal blood sampling of both fetuses was performed. A maternal serum

sample was also obtained at the same time. The zygosity of the twins was determined by inspection of the fetal membranes and DNA analysis.

GC-MS analysis of valproate and its metabolites. Gas chromatographic selective ion monitoring mass spectrometry (GC-SIM-MS) analysis was performed according to Fisher *et al.*(10). Extraction procedure: A sample of 200 μl serum or amniotic fluid or 30 μl (for valproate-glucuronide determination) and 100 μl of urine (for the metabolites) was pipetted into a disposable 1.5 ml Eppendorf microtube. 50 μl of 1N NaH_2PO_4 buffer pH 5.0, and 1 ml of ethylacetate containing 1 $\mu g.ml^{-1}$ 2-ethyl-2-methylcaproic acid (EMCA) as internal standard were added. The tube was shaken for 15 min and centrifuged for 1 min in a 5012 Eppendorf centrifuge. 800 μl of the supernatant was transferred to a 1.5 ml disposable glass vial and concentrated to approximately 200 μl by a stream of nitrogen at 20°C. Then 100 μl of acetonitrile was added. The extraction was repeated with an additional 1 ml of ethylacetate without EMCA. The combined extracts were evaporated to a final volume of 10-20 μl. Trimethylsilylation was accomplished by addition of 30 μl of pyridine and 30 μl of N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) at room temperature for at least 30 min and 1 μl aliquots were injected in splittless mode into the GC-MS system. For deglucuronidation of the urine and removal of urea 50 μl of 1N NaH_2PO_4 (pH 5.0), 30 μl of ß-glucuronidase-arylsulfatase (5 $U.ml^{-1}$) and 10 μl of urease were added. This mixture was slowly agitated for 1 h at 37°C and further processed as described above. Unbound valproate was measured after ultrafiltration of 500 μl of serum with the Amicon MPS-1 Ultrafiltration device (YMT-membrane, cut off 30 kDa). The frozen serum samples were thawed and then centrifuged in a fixed-angle rotor at 1500 x *g* (4000 rpm) for 15 min (Sorvall R C-5B centrifuge) at 37°C.

GC-SIM-MS analysis was carried out using a Perkin-Elmer F22-9C, coupled via a Jet separator to a Finnigan MAT CH-7A mass spectrometer operated by a 2100D Superincos. A fused silica Megabore column was used (30 m x 0.53 mm I.D., 1 μm film thickness), coated with DB 1701 (Carlo Erba Instruments, Hofheim, Germany). The temperature of the injector was held at 220°C. The initial oven temperature was 80°C. After injection, the temperature was held at 80°C for 1 min, rapidly raised to 120°C, and then at a rate of $4°C.min^{-1}$ to 190°C. Detection took place in the selective ion monitoring mode, with the following ions; m/z 183 3-keto-valproate, m/z 185 5-OH-valproate and 2-n-propyl-glutaric acid, m/z 191 3-OH-valproate, m/z 195 3-keto-$\Delta^{4'}$-valproate, m/z 197 (2E,3'E)$\Delta^{2,3'}$-valproate, (E)$\Delta^{2,4}$-valproate, m/z 199 (Z)Δ^{2}-valproate, (E)Δ^{2}-valproate, Δ^{3}-valproate, Δ^{4}-valproate and 4-OH-valproate, m/z 201 valproate and m/z 215 4-keto-valproate and EMCA.

Calibration graphs were linear in the concentration range 0.1-20 $\mu g.ml^{-1}$ for most of the metabolites and up to 150 $\mu g.ml^{-1}$ for valproate. Within- and between-day coefficients of variation were less then 6% in these ranges. The regression coefficients always exceeded 0.99 and the extraction yields for all substances exceeded 90%. The lowest limit of detection of this method is in the low nanogram range.

For statistical analysis the SPSS-PC+4.0 statistical package was used. Pearson's correlation coefficients were used to describe linear relationships; for fitting a line through the data points the method of least squares was used. Arithmetical means and

standard deviations were calculated for the analytical data, whereas for the ratios of the concentrations in amniotic fluid-to-maternal serum the median and range were more appropriate. The Mann-Whitney U test was used to test for differences between groups (monotherapy *vs.* combinationtherapy, and pregnancies with spina bifida outcome *vs.* normal outcome), whereas for pair-wise comparison between two serum samples from the same patient the Wilcoxon matched pair signed ranks test was used. When the 2-tailed $P<0.05$ the difference was considered significant.

2.6.3 Results

Between November 1, 1985 and July 1, 1990, 92 pregnancies (in 81 women) in which valproate was used, alone or in combination with other antiepileptic drugs, were referred for prenatal diagnosis. No woman contributed more than 2 pregnancies to the study. Four of these 92 pregnancies involved twins. In 6 fetuses spina bifida was diagnosed prenatally by ultrasound and raised α-fetoprotein concentrations in the amniotic fluid. Five fetuses (including one pair of twin, both fetuses affected) were exposed to valproate monotherapy and one fetus to a combination of valproate and carbamazepine (table 2.6.1).

Table 2.6.1 Frequency of prenatally diagnosed spina bifida aperta after first trimester exposure to valproate

Exposure to valproate as	Total number of exposed fetuses	Number of fetuses without SBA	Number of fetuses with SBA	%
monotherapy	63 (60)	58 (56)	5 (4)*	7.9 (6.7)
polytherapy	33 (32)	32 (31)	1 (1)	3.0 (3.1)
Total	96 (92)	90 (87)	6 (5)	6.3 (5.4)

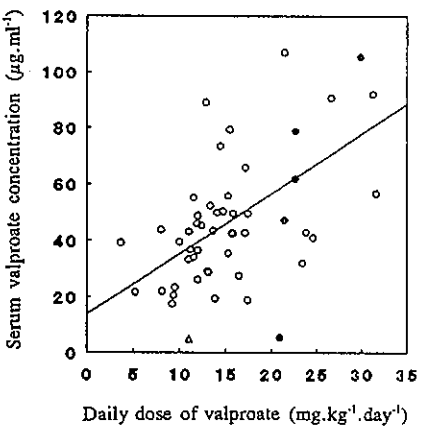
The numbers in parentheses represent pregnancies, twin pairs counted as one. SBA = spina bifida aperta
* including a monozygotic twin pair, concordant for lumbosacral spina bifida aperta.

52 of the 92 pregnancies were included in this study. In 4 women amniocentesis was not carried out, and only a structural ultrasound was obtained. Of 12 women (all singleton pregnancies) informed consent was not obtained. In these cases the amniotic fluid was only used for α-fetoprotein determination. From 8 women amniotic fluid was obtained, but without additional maternal serum or a 24 h urine sample. Another 16 pregnancies did not contribute to this part of the study, either because the women had stopped taking valproate after they had become aware of their pregnancy (6 to 8 weeks of gestation) ($n=11$), or because concentrations of valproate and its metabolites could not be detected in any of the available samples ($n=5$). 40 of the 52 women in this study also provided a serum sample at the intake visit.

Maternal urine. In 24 h urine samples only 34% ($\pm$ 18%) of the daily dose was recovered (table 2.6.2). The major urinary metabolites were the glucuronide conjugate of valproate as well as 3-keto-valproate. Less then 1% of the dose was recovered as unchanged valproate. Besides the glucuronide of valproate, the unsaturated metabolites

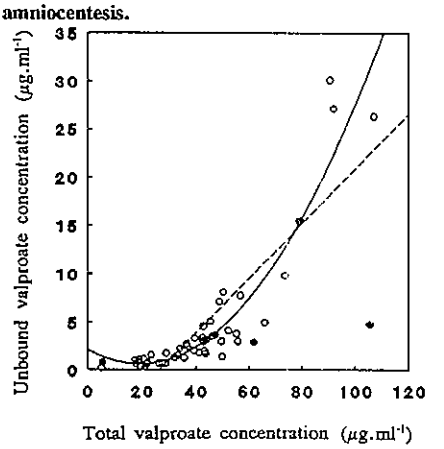
(E)Δ^2-valproate, (Z)Δ^2-valproate, (2E,3'E)$\Delta^{2,3'}$-valproate and $\Delta^{2,4}$-valproate were also present in the urine in the conjugated forms. Δ^3-valproate and Δ^4-valproate were rarely detected, with or without glucuronidase treatment. Total recovery of valproate and its metabolites tended to increase with increasing 24 h urine volumes.

Fig 2.6.1 Correlation between dosage and total concentration of valproate in maternal serum, obtained before amniocentesis.



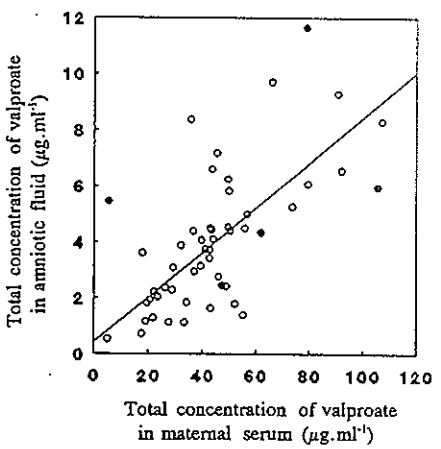
• spina bifida cases; y = 13.87 + 2.13x, r= 0.59 (data points below 6 μg.ml⁻¹ belonged to patients with doubtful treatment compliance and were therefore not included in the regression equation)

Fig 2.6.2 Correlation between total and unbound concentrations of valproate in maternal serum, obtained before amniocentesis.



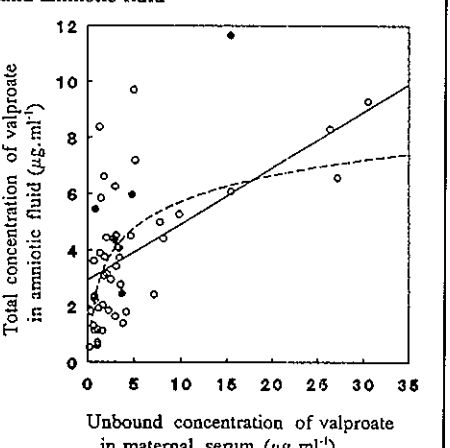
• spina bifida cases; y = -7.51 + 0.28x, r=0.86

Fig 2.6.3 Correlation between total valproate concentrations in maternal serum and amniotic fluid



• spina bifida aperta cases; y = 0.42 + 0.08x, r = 0.72

Fig 2.6.4 Correlation between unbound valproate concentrations in maternal serum and amniotic fluid



• spina bifida aperta cases; y = 2.93 + 0.20x, r = 0.59
(y = 2.63 + 1.34ln(x), r = 0.63)

Table 2.6.2 24 h urinary recovery of valproate and its metabolites, grouped according to the four main metabolic routes of valproate (M ± SD).

Metabolic route	Valproate alone n=35 (% of the dose)	Valproate in combination n=17 (% of the dose)	Total n=52 (% of the dose)	p-value
Total recovery	33 ± 19	35 ± 15	34 ± 18	0.98
VPA-Glucuronide	17 ± 10	22 ± 10	18 ± 10	0.15
ß-oxidation	12 ± 11	11 ± 9	12 ± 10	0.70
((E)Δ^2-VPA-Glu &	0.9 ± 0.5	0.4 ± 0.3		0.001
3-OH-VPA &	0.5 ± 0.5	0.05 ± 0.04		0.001
3-keto-VPA)	11 ± 10	11 ± 8		0.78
ω-oxidation	0.6 ± 0.8	0.6 ± 0.6	0.6 ± 0.7	0.10
(5-OH-VPA &	0.4 ± 0.4	0.3 ± 0.3		0.97
PGA)	0.2 ± 0.4	0.2 ± 0.3		0.29
(ω-1)-oxidation	1.4 ± 1.5	0.6 ± 0.6	1.1 ± 1.3	0.53
(4-OH-VPA &	0.6 ± 0.6	0.3 ± 0.3		0.28
4-keto-VPA)	0.8 ± 0.9	0.3 ± 0.3		0.04
rest group				
(Z)Δ^2-VPA-Glu	0.8 ± 0.5	0.9 ± 0.6		0.54
(E)$\Delta^{2,4}$-VPA-Glu	0.2 ± 0.2	0.10 ± 0.1		0.14
(2E,3'E)$\Delta^{2,3'}$-VPA-Glu	0.9 ± 0.8	0.6 ± 0.6		0.20
	(% of recovered dose)	(% of recovered dose)	(% of recovered dose)	
VPA-Glucuronide	55 ± 22	62 ± 19	57 ± 21	0.37
ß-oxidation	34 ± 20	31 ± 19	33 ± 20	0.74
((E)Δ^2-Glu &	3.0 ± 1.5	1.1 ± 0.5		<0.001
3-OH-VPA &	1.2 ± 1.0	0.1 ± 0.1		<0.001
3-keto-VPA)	30 ± 21	30 ± 19		0.81
ω-oxidation	1.5 ± 1.1	1.7 ± 1.3	1.5 ± 1.2	0.79
(5-OH-VPA &	1.0 ± 0.6	1.0 ± 0.7		0.97
PGA)	0.5 ± 0.6	0.7 ± 0.8		0.32
(ω-1)-oxidation	3.5 ± 2.4	1.6 ± 1.3	2.9 ± 2.2	0.01
(4-OH-VPA &	1.6 ± 1.1	0.9 ± 0.7		0.08
4-keto-VPA)	1.9 ± 1.4	0.7 ± 0.6		0.002
rest group				
(Z)Δ^2-VPA-Glu	2.9 ± 1.7	2.4 ± 1.5		0.43
(E)$\Delta^{2,4}$-VPA-Glu	0.6 ± 0.4	0.3 ± 0.2		0.01
(2E,3'E)$\Delta^{2,3'}$-VPA-Glu	2.9 ± 1.8	1.5 ± 1.2		0.005

VPA = valproate, 2-n-propyl-pentanoic acid; (E)Δ^2-VPA = 2-n-propyl-(E)2-pentanoic acid; (Z)Δ^2-VPA = 2-n-propyl-(Z)2-pentanoic acid, Δ^3-VPA = 2-n-propyl-3-pentanoic acid; Δ^4-VPA = 2-n-propyl-4-pentenoic acid; (2E,3'E)$\Delta^{2,3'}$-VPA = 2-[(E)-1'-propenyl]-(E)3'-pentenoic acid; (E)$\Delta^{2,4}$-VPA = 2-n-propyl-(E)2,4-pentadienoic acid; 3-keto-VPA = 2-n-propyl-3-oxo-pentanoic acid; 4-keto-VPA = 2-n-propyl-4-oxo-pentanoic acid; 3-OH-VPA = 2-n-propyl-3-hydroxy-pentanoic acid; 4-OH-VPA = 2-n-propyl-4-hydroxy-pentanoic acid; 5-OH-VPA = 2-n-propyl-4-hydroxy-pentanoic acid; PGA = 2-n-propyl-glutaric acid

Table 2.6.3 Daily dose and serum concentrations of valproate and its metabolites in serum obtained at intake and before amniocentesis

	Serum obtained at intake (SI) VPA alone ($\mu g.ml^{-1}$) (n=29)	VPA in combination ($\mu g.ml^{-1}$) (n=11)	p-value	Serum obtained before amniocentesis (SA) VPA alone ($\mu g.ml^{-1}$) (n=35)	VPA in combination ($\mu g.ml^{-1}$) (n=17)	p-value	SI-SA pair-wise comparison p-value
VPA daily dose mg	989 ± 418	1109 ± 539	0.65	1050 ± 407	1088 ± 499	0.98	1.0
$mg.kg^{-1}$	14.1 ± 6.3	14.6 ± 6.8	0.95	15.5 ± 6.4	15.3 ± 6.3	0.85	1.0
Metabolite							
VPA	49 ± 24	38 ± 11	0.13	52 ± 25	35 ± 13	0.02	0.98
(E)Δ^2-VPA	2.7 ± 1.2	1.7 ± 0.7	0.006	2.6 ± 1.1	1.5 ± 0.7	0.001	0.04
(Z)Δ^2-VPA	0.3 ± 0.2	0.3 ± 0.1	0.39	0.2 ± 0.2	0.2 ± 0.1	0.74	0.03
Δ^3-VPA	1.0 ± 0.4	0.7 ± 0.3	0.03	0.7 ± 0.3	0.5 ± 0.2	0.002	0.0003
Δ^4-VPA	0.08 ± 0.1	0.1 ± 0.04	0.07	0.1 ± 0.1	0.08 ± 0.08	0.97	0.96
(2E,3'E)$\Delta^{2,3'}$-VPA	1.5 ± 0.6	1.1 ± 0.5	0.03	1.2 ± 0.6	0.9 ± 0.6	0.04	0.0001
(E)$\Delta^{2,4}$-VPA	0.3 ± 0.2	0.4 ± 0.2	0.18	0.3 ± 0.2	0.4 ± 0.2	0.54	0.33
3-keto-VPA	1.5 ± 0.8	2.6 ± 1.3	0.004	1.6 ± 1.1	1.8 ± 1.1	0.36	0.06
4-keto-VPA	0.04 ± 0.06	0.1 ± 0.1	0.04	0.05 ± 0.06	0.1 ± 0.1	0.005	0.16
3-OH-VPA	0.06 ± 0.04	0.01 ± 0.01	0.0002	0.04 ± 0.03	0.004 ± 0.01	0.0001	0.03
4-OH-VPA	0.09 ± 0.05	0.1 ± 0.05	0.49	0.09 ± 0.05	(0.1) ± 0.08	0.25	0.87
5-OH-VPA	0.06 ± 0.03	0.07 ± 0.04	0.20	0.06 ± 0.03	0.07 ± 0.03	0.18	0.81
PGA	0.00 ± 0.00	0.02 ± 0.05	0.02	0.00 ± 0.00	0.03 ± 0.08	0.009	1.00

The data are shown as mean ± SD; VPA = valproate, 2-n-propyl-pentanoic acid; (E)Δ^2-VPA = 2-n-propyl-(E)2-pentanoic acid; (Z)Δ^2-VPA = 2-n-propyl-(Z)2-pentanoic acid, Δ^3-VPA = 2-n-propyl-3-pentanoic acid; Δ^4-VPA = 2-n-propyl-4-pentenoic acid; (2E,3'E)$\Delta^{2,3'}$-VPA = 2-[(E)-1'-propenyl]-(E)3'-pentenoic acid; (E)$\Delta^{2,4}$-VPA = 2-n-propyl-(E)2,4-pentadienoic acid; 3-keto-VPA = 2-n-propyl-3-oxo-pentanoic acid; 4-keto-VPA = 2-n-propyl-4-oxo-pentanoic acid; 3-OH-VPA = 2-n-propyl-3-hydroxy-pentanoic acid; 4-OH-VPA = 2-n-propyl-4-hydroxy-pentanoic acid; 5-OH-VPA = 2-n-propyl-4-hydroxy-pentanoic acid; PGA = 2-n-propyl-glutaric acid

Maternal serum. Figure 2.6.1 shows the correlation between the daily dose of valproate and valproate concentrations in maternal serum. Valproate, (E)Δ^2-valproate, 3-keto-valproate and (2E,3'E)$\Delta^{2,3'}$-valproate were detected in the highest concentrations; the serum concentrations of the more hydrophilic hydroxylated metabolites were much lower. Concentrations of 3-OH-valproate and 2-n-propyl-glutaric acid were often below the detection limit. Only very low concentrations of Δ^4-valproate were found and it was not detectable in all samples. Unbound valproate serum concentrations around 16 weeks of gestation varied from 0.8 to 33.7 $\mu g.ml^{-1}$, corresponding to an unbound fraction of 0.2% to 27.2%. All unbound unsaturated metabolites were below the detection limit. 3-keto-valproate, 4-keto-valproate, the hydroxy-metabolites and 2-n-propyl-glutaric acid were hardly protein bound. The unbound fraction increases disproportionately with increasing serum valproate concentrations (fig. 2.6.2).

Table 2.6.4 Medians of ratios and Pearson's correlation coefficients of the concentrations of valproate and its metabolites in amniotic fluid (AF) and the total (SA) concentration or unbound concentration (UBSA) in maternal serum

Compound	Correlation AF *vs.* SA		Ratio AF/SA		Ratio AF/UBSA	
	r	p-value	Median	(range)	Median	(range)
VPA	0.61	<0.0000	0.09	(0 - 0.2)	1.3	(0-6.6)
(E)Δ^2-VPA	0.73	<0.0000	0.04	(0 - 0.1)		
(Z)Δ^2-VPA	0.14	0.34	0	(0 - 0.4)		
Δ^3-VPA	0.30	0.03	0	(0 - 0.4)		
Δ^4-VPA	0.03	0.81	0	(0 - 0.3)		
(2E,3'E)-$\Delta^{2,3'}$-VPA	0.59	<0.0000	0.04	(0 - 0.1)		
(E)-$\Delta^{2,4}$-VPA	0.34	0.02	0.07	(0 - 0.2)		
3-keto-VPA	0.53	0.0001	0.2	(0 - 1.1)	0.3	(0.02-1.4)
4-keto-VPA	0.33	0.02	0.5	(0 - 3.4)	0.3	(0-2.2)
3-OH-VPA	-0.02	0.87	0.0	(0 - 0.7)		
4-OH-VPA	0.36	0.01	0.4	(0 - 1.4)	0.3	(0-1.7)
5-OH-VPA	0.28	0.05	0.5	(0 - 2.0)		
PGA	-0.05	0.75	-	-		

VPA = valproate, 2-n-propyl-pentanoic acid; (E)Δ^2-VPA = 2-n-propyl-(E)2-pentanoic acid; (Z)Δ^2-VPA = 2-n-propyl-(Z)2-pentanoic acid, Δ^3-VPA = 2-n-propyl-3-pentanoic acid; Δ^4-VPA = 2-n-propyl-4-pentenoic acid; (2E,3'E)$\Delta^{2,3'}$-VPA = 2-[(E)-1'-propenyl]-(E)3'-pentenoic acid; (E)$\Delta^{2,4}$-VPA = 2-n-propyl-(E)2,4-pentadienoic acid; 3-keto-VPA = 2-n-propyl-3-oxo-pentanoic acid; 4-keto-VPA = 2-n-propyl-4-oxo-pentanoic acid; 3-OH-VPA = 2-n-propyl-3-hydroxy-pentanoic acid; 4-OH-VPA = 2-n-propyl-4-hydroxy-pentanoic acid; 5-OH-VPA = 2-n-propyl-4-hydroxy-pentanoic acid; PGA = 2-n-propyl-glutaric acid

A comparison of the serum concentrations of valproate and its metabolites between monotherapy and combination-therapy showed higher concentrations of (E)Δ^2-valproate, Δ^3-valproate, (2E,3'E)$\Delta^{2,3'}$-valproate and 3-OH-valproate in serum of women treated with valproate alone (table 2.6.3, Mann-Whitney U-test). Concentrations of 3-keto-valproate and 4-keto-valproate were higher in the combination-therapy group, and 2-n-propyl-glutaric acid could only be detected in the serum of some patients on combination-therapy. Pair-wise comparison showed significantly higher concentrations of (E)Δ^2-valproate, (Z)Δ^2-valproate, Δ^3-valproate, (2E,3'E)$\Delta^{2,3'}$-valproate and 3-OH-valproate in intake serum (gestational age 13.4 $\pm$ 2.2 weeks) compared with serum taken prior to

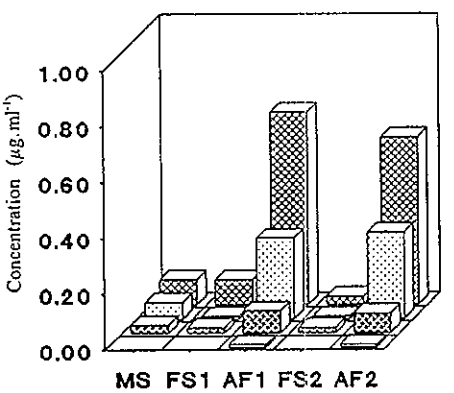
amniocentesis (gestational age 16.7 ± 0.7 weeks) (table 2.6.3, last column SI-SA, Wilcoxon matched-pairs signed-ranks test).

Amniotic fluid. In amniotic fluid valproate concentrations varied from less than 0.01 to 11.7 $\mu g.ml^{-1}$. Valproate concentrations in amniotic fluid correlated better with total than unbound serum concentrations (fig. 2.6.3 and 2.6.4), due to the extremely high unbound fractions observed when serum concentrations exceeded 90 $\mu g.ml^{-1}$. The unsaturated metabolite (E)Δ^2-valproate was present in amniotic fluid in concentrations from below 0.01 to 0.3 $\mu g.ml^{-1}$, always much lower than the total concentrations in maternal serum (0.3 to 5.9 $\mu g.ml^{-1}$).

The ratios of the concentrations of valproate and its metabolites in amniotic fluid-to-maternal serum are shown in table 2.6.4 as median (range), since they varied strongly. Whereas the concentrations of the more hydrophillic metabolites were up to 50% of those in maternal serum (especially of 4-keto-valproate, 4-OH-valproate and 5-OH-valproate), the unsaturated metabolites could not be detected in most amniotic fluid samples.

It is likely that concentrations of valproate and its metabolites in amniotic fluid lag behind those in serum. This was best demonstrated in amniotic fluid samples (from the affected twin pregnancy) and serum obtained before the termination of pregnancy. The patient reported that she had not taken her medication the day before termination of pregnancy, *i.e.* for more than 36 h (data shown in fig. 2.6.5). While the concentrations in maternal and fetal sera were below 0.1 $\mu g.ml^{-1}$, in the amniotic fluid samples it was still 0.7 and 0.6 $\mu g.ml^{-1}$, respectively. Of the metabolites only (E)Δ^2-valproate, (2E,3'E)$\Delta^{2,3'}$-valproate, 3-keto-valproate and 5-OH-valproate were above the detection limits and all in higher concentrations in amniotic fluid than in maternal and fetal serum.

Fig 2.6.5 Valproate and some of its metabolites in materal and fetal serum and amniotic fluid in an affected twin pregnancy



MS = maternal serum; FS1 or FS2 = serum of fetus 1 or 2; AF1 or AF2 = amniotic fluid surrounding fetus 1 or 2

Valproate (E)Δ^2-valproate 3-keto-valproate 5-OH-valproate

The women carrying a fetus with spina bifida aperta used significantly higher daily doses of valproate. The individual daily doses used were 1700, 1800, 1200, 2000 mg as monotherapy, and 1500 mg valproate in combination with 600 mg carbamazepine, respectively. Furthermore, the mean serum concentrations of these mothers was significantly higher (73 $\mu g.ml^{-1}$) than in other mothers (44 $\mu g.ml^{-1}$) (P=0.02; table 2.6.5). The affected twin pregnancy (1800 $mg.d^{-1}$) was excluded from this analysis, since there was doubt about the compliance during the days before sampling. We observed no significant metabolic differences between pregnancies with a spina bifida aperta and those without (table 2.6.5).

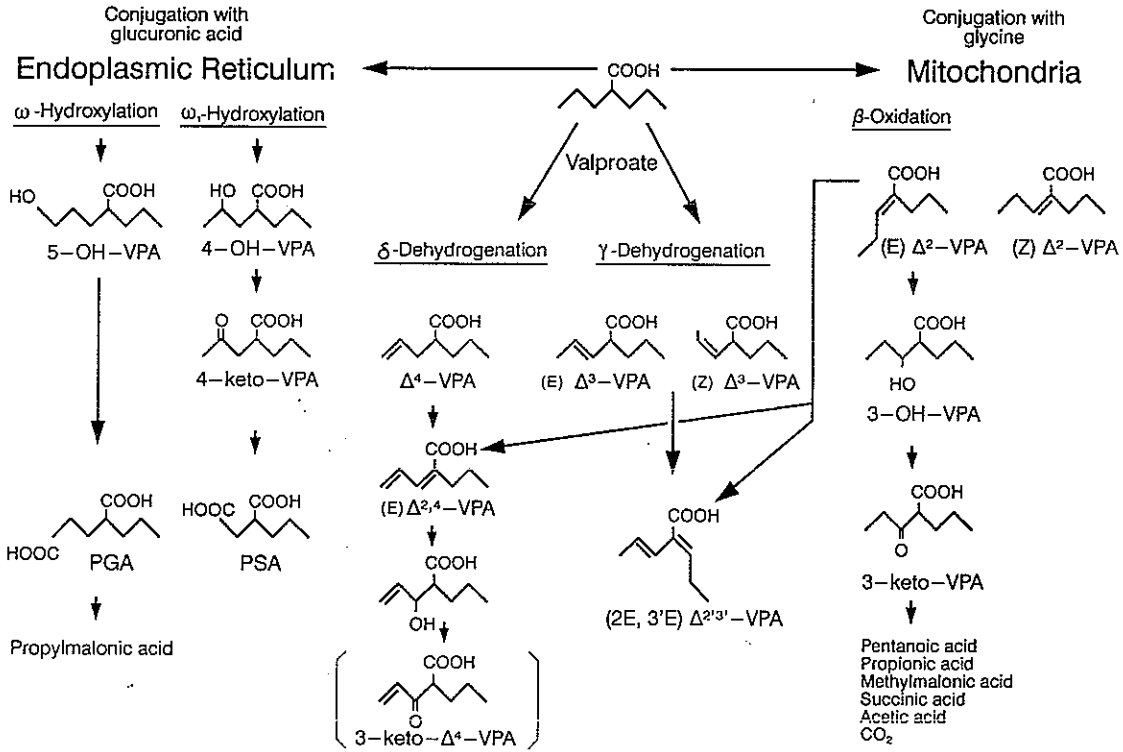
Table 2.6.5 Concentrations of valproate and its metabolites, according to pregnancy outcome

Compound	Normal outcome n=47	spina bifida n=4*	p-value
Valproate daily dose			
$mg.kg^{-1}$	1016 ± 433	1600 ± 337	0.01
$mg.kg^{-1}.d^{-1}$	14.7 ± 5.9	24.1 ± 3.8	0.01
Maternal serum			
Valproate	44 ± 22	73 ± 25	0.02
(E)Δ^2-VPA	2.2 ± 1.1	2.2 ± 0.7	0.96
(Z)Δ^2-VPA	0.2 ± 0.2	0.2 ± 0.2	0.61
Δ^3-VPA	0.7 ± 0.3	0.7 ± 0.2	0.99
Δ^4-VPA	0.1 ± 0.1	0.01 ± 0.01	0.32
(2E,3'E)-$\Delta^{2,3'}$-VPA	1.1 ± 0.6	0.8 ± 0.4	0.38
(E)-$\Delta^{2,4}$-VPA	0.4 ± 0.2	0.3 ± 0.05	0.17
3-keto-VPA	1.7 ± 1.1	0.9 ± 0.6	0.06
4-keto-VPA	0.08 ± 0.09	0.03 ± 0.05	0.17
3-OH-VPA	0.03 ± 0.03	0 ± 0	0.03
4-OH-VPA	0.1 ± 0.06	0.06 ± 0.02	0.29
5-OH-VPA	0.06 ± 0.03	0.05 ± 0.02	0.61
PGA	0.01 ± 0.05	0 ± 0	0.01
Unbound valproate	4.7 ± 6.9	6.7 ± 5.9	0.13
Valproate in amniotic fluid	6.1 ± 4.0	3.6 ± 2.4	0.13
Urinary data			
Total of the dose (%) recovered	33 ± 18	42 ± 9	0.24
	(% of recovered dose)	(% of recovered dose)	
VPA-Glucuronide	56 ± 21	63 ± 7	0.74
ß-oxidation	33 ± 20	30 ± 1	0.93
((E)Δ^2-Glu &	2.3 ± 1.6	3.5 ± 1.2	0.17
3-OH-VPA &	0.9 ± 1.0	0.7 ± 1.0	0.45
3-keto-VPA)	30 ± 21	26 ± 2	0.87
ω-oxidation	1.0 ± 0.7	1.0 ± 0.7	0.93
(5-OH-VPA &	1.0 ± 0.6	1.0 ± 0.7	0.97
PGA)	0.5 ± 0.7	0.4 ± 0.4	0.95
(ω-1)-oxidation	2.9 ± 2.3	2.5 ± 3.6	0.34
(4-OH-VPA &	1.4 ± 1.0	1.4 ± 1.8	0.71
4-keto-VPA)	1.6 ± 1.4	1.1 ± 1.8	0.28
rest group			
(Z)Δ^2-VPA-Glu	2.9 ± 1.6	1.1 ± 1.7	0.12
(E)$\Delta^{2,4}$-VPA-Glu	0.5 ± 0.4	0.2 ± 0.3	0.20
(2E,3'E)$\Delta^{2,3'}$-VPA-Glu	2.6 ± 1.8	1.2 ± 1.2	0.17

* data from the affected twin pregnancy excluded, because of doubts about compliance

VPA = valproate, 2-n-propyl-pentanoic acid; (E)Δ^2-VPA = 2-n-propyl-(E)2-pentanoic acid; (Z)Δ^2-VPA = 2-n-propyl-(Z)2-pentanoic acid, Δ^3-VPA = 2-n-propyl-3-pentanoic acid; Δ^4-VPA = 2-n-propyl-4-pentenoic acid; (2E,3'E)$\Delta^{2,3'}$-VPA = 2-[(E)-1'-propenyl]-(E)3'-pentenoic acid; (E)$\Delta^{2,4}$-VPA = 2-n-propyl-(E)2,4-pentadienoic acid; 3-keto-VPA = 2-n-propyl-3-oxo-pentanoic acid; 4-keto-VPA = 2-n-propyl-4-oxo-pentanoic acid; 3-OH-VPA = 2-n-propyl-3-hydroxy-pentanoic acid; 4-OH-VPA = 2-n-propyl-4-hydroxy-pentanoic acid; 5-OH-VPA = 2-n-propyl-4-hydroxy-pentanoic acid; PGA = 2-n-propyl-glutaric acid

Fig 2.6.6 Tentative metabolic scheme of valproate metabolism in humans



VPA = valproate, 2-n-propyl-pentanoic acid; (E)Δ^2-VPA = 2-n-propyl-(E)2-pentanoic acid; (Z)Δ^2-VPA = 2-n-propyl-(Z)2-pentanoic acid, Δ^3-VPA = 2-n-propyl-3-pentanoic acid; Δ^4-VPA = 2-n-propyl-4-pentenoic acid; (2E,3'E)$\Delta^{2,3'}$-VPA = 2-[(E)-1'-propenyl]-(E)3'-pentenoic acid; (E)$\Delta^{2,4}$-VPA = 2-n-propyl-(E)2,4-pentadienoic acid; 3-keto-VPA = 2-n-propyl-3-oxo-pentanoic acid; 4-keto-VPA = 2-n-propyl-4-oxo-pentanoic acid; 3-OH-VPA = 2-n-propyl-3-hydroxy-pentanoic acid; 4-OH-VPA = 2-n-propyl-4-hydroxy-pentanoic acid; 5-OH-VPA = 2-n-propyl-4-hydroxy-pentanoic acid; PSA = 2-n-propyl-succinic acid; PGA = 2-n-propyl-glutaric acid

2.6.4 Discussion

Based on current knowledge of the metabolism of valproate in humans, valproate metabolites have been grouped into those resulting from four major pathways (glucuronidation, ß-oxidation, ω- and (ω-1)-oxidation) and three additional minor pathways (γ- and δ-dehydrogenation and conjugation with glycine)(11) (fig. 2.6.6). In vitro data (12) have suggested that both 3-OH-valproate and 3-keto-valproate may also be produced by a pathway which does not involve ß-oxidation. However, studies of the metabolism of valproate are problematic. Some of the above-mentioned pathways frequently interconnect with each other or with intermediary metabolism; factors such as dietary protein and fat intake and free fatty acid concentrations influence valproate metabolism by competing with ß-oxidation; strict mass balance accounting of metabolites from urinary data is further hindered by the lability and volatility of some metabolites and the existence of non-renal excretory routes (12, 14, 16).

The urinary data, expressed as a percentage of the recovered dose, were in general agreement with the results of previous studies (14, 15). However, in this study the mean total recovery of the daily dose in 24 h urine samples was much lower than others have reported (14, 15). Although incomplete urine collection and patient compliance problems can not be excluded, this lower total recovery is mainly caused by lower concentrations of valproate-glucuronide. Except for (ω-1)-oxidation, no significant differences were found between urinary metabolite patterns of patients on monotherapy versus combination-therapy.

Although serum concentrations were not always collected in the same dosing interval or before the morning dose, we found significant correlations between daily dose and serum valproate concentrations. Since especially factors like gender, age and combination-therapy cause wide variations in serum concentrations, the relatively good correlation is probably due to the homogeneity of the study group, involving only women at a specific stage of pregnancy and relatively many taking valproate only. The mean serum concentration of valproate was lower in patients on combination-therapy. Comedication also led to significantly lower concentrations of ß-oxidation products ((E)Δ^2-valproate, Δ^3-valproate, and (2E,3'E)$\Delta^{2,3'}$-valproate). The lower serum concentrations of ß-oxidation metabolites of valproate in early second trimester compared with late first trimester, also suggests a reduction in ß-oxidation with progressing pregnancy. Free fatty acids tend to increase during gestation and high concentrations of fatty acids can compete with valproate for ß-oxidation (6, 16).

Both the mother and the fetus make individual contributions to the amniotic fluid and the extent of this varies at different stages of pregnancy. From 12 to 14 weeks the fetus starts to produce urine, and excretes it into the amniotic fluid (17). Therefore, there may have been a small contribution of fetal urine. The higher amniotic fluid-to-maternal serum ratio for the hydroxylated metabolites compared with the more lipophilic metabolites might have reflected this fetal contribution. Rettie and coworkers (18) have shown that hydroxylation of valproate (3-OH-valproate, 4-OH-valproate, 5-OH-valproate) occurred in vitro in homogenates of tissues from aborted human fetuses of 50-72 days of

age, particularly in fetal adrenal tissue and to a lesser extent in fetal liver. Products of ß-oxidation (principally 3-keto-valproate) were also occasionally, but inconsistently, detected. In all the tissue homogenates studied, hydroxylation occurred principally at the 4-position. However, preferential partition of the hydroxylated metabolites in the more watery amniotic fluid might also be an explanation for the higher amniotic fluid-to-maternal serum ratios for the hydroxylated metabolites.

Our data showed further that the concentrations of valproate and its metabolites in amniotic fluid lagged behind those in maternal serum. Dickinson (19) has shown in the rhesus monkey that after the administration of valproate to the mother the concentration of valproate in amniotic fluid rose only slowly and approached the concentration present in maternal and fetal blood at about 180 min. However, when valproate was administered to the fetus, valproate rapidly appeared in the amniotic fluid and in the maternal circulation. Our data from a patient who skipped several doses before amniocentesis showed that valproate remained longer in amniotic fluid than in maternal serum, thereby suggesting that amniotic fluid acts as a deep compartment.

Although doses and maternal serum concentrations of valproate were significantly higher in affected pregnancies, we found no consistent differences in metabolite pattern maternal serum, 24 h urine samples, or amniotic fluid. The wide variation in the patterns of urinary metabolite excretion and inter- and intra-individual fluctuations in serum and amniotic fluid profiles preclude the use of these data as a basis for detecting minor differences in metabolite concentrations. Studies in the mouse in vivo showed that of the metabolites tested, only the teratogenicity of Δ^4-valproate was very similar to that of valproate, while Δ^2-valproate, $\Delta^{4,4'}$-valproate and 2-n-propyl-glutaric acid showed less exencephaly at the same dose levels (20). Since, the metabolite concentrations in human serum and amniotic fluid were all much lower than valproate, it is not very likely that the teratogenic effect is caused by one of the measured metabolites. Moreover, Δ^4-valproate, was lower rather than higher in the pregnancies with spina bifida.

In conclusion, the higher dosage used in affected pregnancies, resulting in higher serum concentrations of valproate is in agreement with the hypothesis that the increased risk for spina bifida is due to the parent drug (20, 21, 22).

2.6.5 References

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THE 10,11-EPOXIDE - 10,11-DIOL PATHWAY OF CARBAMAZEPINE IN EARLY PREGNANCY IN MATERNAL SERUM, URINE AND AMNIOTIC FLUID: EFFECT OF DOSE, COMEDICATION AND RELATION TO OUTCOME OF PREGNANCY

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Abstract

Epoxide metabolites of carbamazepine have been suggested to play a role in the occurrence of congenital malformations observed in infants exposed to carbamazepine. We have investigated the 10,11-epoxide - 10,11-diol pathway of carbamazepine in pregnant epileptic patients receiving carbamazepine alone or in combination with other antiepileptic drugs in relation to the outcome of pregnancy. In 100 pregnancies with first trimester carbamazepine exposure (including 7 with malformed outcome) parent drug and metabolite concentrations in maternal serum were evaluated. Carbamazepine-10,11-epoxide concentrations increased with increasing dose. Comedication with phenobarbital led to lower 10-11-epoxide concentrations in maternal serum and a higher percentage of the dose recovered in urine as 10,11-diol. Valproate comedication led to slightly higher 10,11-epoxide concentrations in maternal serum, in combination with lower carbamazepine concentrations and a lower percentage of the dose recovered in the urine as 10,11-diol. In amniotic fluid, concentrations of carbamazepine and its main metabolites were in most patients 2 to 2.5 times higher than the free concentrations in maternal serum. Metabolites and parent drug concentrations in amniotic fluid correlated with their free concentration in maternal serum, but stronger with each other in amniotic fluid.
No significant differences in levels of carbamazepine and its metabolites were observed between pregnancies with normal and malformed outcome. The metabolite to parent drug concentration ratios showed only in amniotic fluid small but significant differences.

2.7.1 Introduction

In general, maternal epilepsy and the use of anticonvulsants is associated with a 2 to 3 fold increase in congenital anomalies in the offspring (1). The prevalence of malformations appears to increase with increasing numbers of antiepileptic drugs used, concomitantly (2, 3). Lindhout and Meinardi (2) searched for specific metabolic interactions between different antiepileptic drugs which could explain this phenomenon. They found the highest malformation frequency (7/12, including 2 cases of dysmorphia and mental retardation) with combinations of carbamazepine and phenobarbital and valproate with or without phenytoin. In a separate study (2), including both male and female patients, they found that with this combination the ratio of areas under the concentration-time (10 h) curves between carbamazepine-10,11-epoxide and carbamazepine was higher than with other combinations or with carbamazepine alone and they suggested that this was due to an accumulation of the 10,11-epoxide. Based on these data they postulated that due to metabolic interaction an increase in the production or decrease in detoxification of reactive epoxide metabolites could be responsible for the high incidence of malformations and they suggested that the carbamazepine-10,11-epoxide to carbamazepine concentration ratio could be indicative for the accumulation of other, potentially more harmful, epoxides as well (2).

To study this hypothesis further, they cultured whole rat-embryos (day 10) *in vitro* in the presence of sera from patients (both males and females) with both low and high carbamazepine-10,11-epoxide to carbamazepine concentration ratios and sera from healthy controls (4). In these experiments, they found that sera from patients induced a lower score for caudal neural tube closure, which correlated with sodium-concentrations ($r=0.58$; $p<0.01$) and with the carbamazepine-10,11-epoxide to carbamazepine ratio ($r=-0.59$; $p<0.02$), whereas there was no correlation with carbamazepine-10,11-epoxide or carbamazepine concentrations as such. Total morphology scores did not correlate with any of these parameters. They concluded that these data supported their previous hypothesis, although neural tube defects were not observed in the above mentioned study (2). Only recently, however, first trimester carbamazepine exposure was associated with a risk of neural tube defects of about 0.9% of exposed infants and this is about 10 times higher than the rate in the background population (5). Furthermore, Jones and his colleagues (6) described a high frequency of minor anomalies, like craniofacial defects, fingernail hypoplasia and developmental delay after carbamazepine exposure in utero.

Clearly, carbamazepine is an example of an antiepileptic drug whose teratogenic potential has not as yet been fully elucidated. Nevertheless, it is repeatedly suggested that the 10,11-epoxide might be the potential teratogenic agent (6, 7). Therefore, we feel the need to describe our experience in a prospective cohort over a period of almost 5 years, in which carbamazepine was the drug most frequently used (50%), either as monotherapy or polytherapy. We gave special attention to the 10,11-epoxide - 10,11-diol pathway and the occurrence of hyponatraemia in the same pregnant patients as from whom we evaluated pregnancy outcome. Since all patients entered the study for prenatal diagnosis by means of amniocentesis (at 16-20 weeks of gestation, without prior knowledge on fetal

outcome) we were also able to determine to what extent carbamazepine and its main metabolites reach the amniotic fluid. Since in early pregnancy amniotic fluid is considered an extension of the fetal extracellular space (8), we were interested whether amniotic fluid could provide useful fetal exposure data.

2.7.2 Methods

Clinical protocol. The protocol was approved by the Medical Ethics Committee of the Erasmus University Rotterdam and the University Hospital Rotterdam - Dijkzigt. The study population consisted of pregnant epileptic women using antiepileptic drugs. Subjects were referred to the outpatient clinic for prenatal diagnosis of neural tube defects and other major malformations by amniocentesis at 16-18 weeks of gestation and/or structural ultrasound examination at 18-22 weeks of gestation. After informed consent was obtained an interview was taken, including among other things questions on drug regimen (formulation, dose and dosing schedule) before and during pregnancy and other maternal exposures. The participants were assured that the medication should be continued as prescribed by the neurologist and were asked to report at amniocentesis whether there had been any change in medication during the interval between intake and amniocentesis.

Gestational age was calculated from the first day of the last menstrual period and from ultrasonic measurements of crown-rump length or biparietal diameter. Birth measurements were plotted on the growth charts of Usher and McLean (8). Infants with birth measurements below the 3rd centile were categorized as small for gestational age or in case of head circumference as microcephalic. Only major congenital abnormalities or malformations were included and only if the diagnosis was made within the first three months of life or suspected and confirmed by diagnostic procedures initiated within this period.

Sample collection and storage. Blood samples were obtained randomly within the dosing-interval at the first consultation (6-14 weeks of gestation), and directly prior to the amniocentesis (16-20 weeks of gestation). After clotting the blood sample was centrifuged for 10 min at 2000 RPM and after separation the serum was frozen and stored at -80°C until analysis. The day prior to amniocentesis the patient herself collected urine for 24 h and stored it in the refrigerator. On the day of amniocentesis the 24 h urine volume was determined and a sample was stored at -80°C until analysis. The amount of amniotic fluid collected was as usual for prenatal diagnosis, being 10 to 20 ml, and after α-fetoprotein determination the remaining supernatant was stored at -80°C.

Chemicals. Carbamazepine (CBZ), carbamazepine-10,11-epoxide (10,11-epoxide) and *trans*-10,11-dihydro-10,11-dihydroxy-carbamazepine (10,11-diol) were all gifts from Dr. R. Heckendorn of Ciba-Geigy (Basel, Switzerland). Hexobarbital served as the internal standard and was purchased from Sigma Chemical Co. (St. Louis, MO USA). ß-glucuronidase-arylsulfatase (crude solution from Helix pomatia, Type H-2) was also purchased from Sigma Chemical Co. HPLC grade acetonitrile and methanol were purchased from LAB Scan Limited Co. (Dublin, Ireland). Water was destillated twice,

filtered through Millipore HVLP filters (type HV-LP, pore size 0.45 μm) and degassed with helium. Sodium dihydrogen phosphate, sodium acetate, acetic acid and ethylacetate (all pro analysi quality) were purchased from Merck (Darmstadt, Germany).

Analytical procedure. The extraction procedures for serum, amniotic fluid and ultrafiltrate were according to Wad (9). To 200 μl serum, 200 μl saturated phosphate buffer (pH=3.4) and 300 μl acetonitrile, containing hexobarbital as the internal standard, were added in a 1.5 ml Eppendorf tube. For serum analysis the hexobarbital concentration was 34.6 $\mu g.ml^{-1}$. This solution was diluted 5 times (final concentration 6.9 $\mu g.ml^{-1}$) for the analysis of amniotic fluid and ultrafiltrate. Ultrafiltrate was prepared from 500 μl serum, using YMT-membranes in the MPS-1 Micropartition System (Amicon BV, Oosterhout, The Netherlands).

For determination of total carbamazepine and its metabolites in urine (both unbound and conjugated to glucuronic acid), 100 μl aliquots of pooled 24 h urine samples, to which 0.4 ml acetate buffer (0.5 M, pH=5.0) and 10 μl ß-glucuronidase (≈ 1100 U) were added, were incubated aerobically overnight at 37°C in a 2 ml Eppendorf tube to allow complete deglucuronidation. The next morning 1.5 ml ethylacetate, containing the internal standard (IS) hexobarbital (12.7 $\mu g.ml^{-1}$), was added. The mixture was vortexed for 30 s, rotated for 15 min and centrifuged for 5 min in an Eppendorf centrifuge. The organic phase was transferred to an 1.5 ml injection vial and evaporated to dryness under a gentle stream of nitrogen at 37°C. In the meantime a second extraction step was conducted with another 1.5 ml ethylacetate without IS and the extracts were combined. The residue was reconstituted in 500 μl acetonitrile.

Standard curves for carbamazepine and its metabolites were established by adding known amounts of each compound to drug-free serum, amniotic fluid, physiological buffer solution or urine, respectively. For serum and amniotic fluid concentrations ranged from 0.5 to 10 $\mu g.ml^{-1}$ for the metabolites and 0.9 to 15 $\mu g.ml^{-1}$ for carbamazepine. For ultrafiltrate the concentration ranges used were 1 to 5 $\mu g.ml^{-1}$ for all compounds. In urine the concentration ranges used for the standard curves were 1 to 100 $\mu g.ml^{-1}$ for carbamazepine and the 10,11-epoxide and up to 250 $\mu g.ml^{-1}$ for 10,11-diol. Over these ranges the standard curves exhibited linearity. Recoveries from serum, ultrafiltrate, amniotic fluid and urine all exceeded 97%. The detection limit (detection response four times the noise level) for carbamazepine and its 10,11-epoxide was 0.01 $\mu g.ml^{-1}$ and for 10,11-diol 0.02 $\mu g.ml^{-1}$. Calibration curves were determined daily by a three points linear regression analysis. The correlation coefficients always exceeded 0.99. The within day variation was less than 3% and between days variation was always less than 7% for all compounds. The concentrations of carbamazepine and its metabolites were determined from the standard curves by calculating the ratio of the peak areas of each compound to the peak area of the internal standard.

The HPLC system (LKB-Produkter AB, Bromma, Sweden) consisted of 2 pumps (model 2150), a controller (model 2152), a solvent conditioner (model 2156) and a variable wavelength monitor (model 2141) set at 214 nm. The detector was connected by an interface (760 series, from Nelson analytical, Inc.) to a personal computer with Nelson software (model 2600 Chromatography software, rev. 5.0). With a Promis II HPLC auto-

injector (Spark Holland, BV, Emmen, The Netherlands) 20 μl of the extracts were applied to the column (250 x 4.6 mm, (5 μm) Spherisorb ODS-2, Chrompack International BV, Middelburg, The Netherlands). As mobile phase acetonitrile:water (27:73) was used with a flow rate of 1 ml.min^{-1}. Concomitant use of valproic acid, ethosuximide, primidone, phenobarbital and phenytoin did not interfere with this assay.

Statistical evaluation. Level-dose relationships were fitted by linear regression and were considered significant if $p<0.0001$. Oneway analysis of variance was used to test for differences, with Tukey's test for multiple comparisons with a significance level of 0.05.

2.7.3 Results

Within the study period (November 1, 1985 until July 1, 1990) 159 pregnancies of 137 different women, with carbamazepine use in the first trimester, entered the study. Nine of these 159 pregnancies ended with the birth of a malformed infant (table 2.7.1), of which two were terminated after prenatal diagnosis (cases nos. 1 and 7). In 100 (7 with malformed offspring) of these 159 pregnancies, concentrations of carbamazepine, 10,11-epoxide and 10,11-diol were determined in maternal serum and amniotic fluid. Reasons for exclusion of the other 59 were: lack of an obtained informed consent (n=21); only a structural ultrasound without amniocentesis was performed (n=14); amniotic fluid was obtained, but without additional maternal serum (n=15); carbamazepine therapy (n=2) or comedication (n=2) was changed to other antiepileptic drugs between 8 and 16 weeks of

Table 2.7.1 Congenital anomalies in combination with first trimester carbamazepine use

No.	Type of congenital anomalies	DPH	PHB	PMD	CBZ	VPA	CLP	CLM	♂/♀
7.	Hypospadia, hydrocephalus, cleft lip and palate	-	-	-	200	-	-	-	♂
11.	Club foot left	-	-	-	600	-	-	-	♀
16.	Hypospadia, ptosis (S.Horner)	-	-	-	600	-	-	-	♂
17.	Hypertrophic pylorusstenosis	-	-	-	400	-	-	-	♂
1.	Meningocele with hydrocephalus*	-	-	-	600	1500	-	-	♀
9.	Cleft palate (soft total, hard 1/3)	-	-	-	800	1200	-	-	♀
13.	Microcephaly (<-3 SD)	-	-	750	600	-	-	30	♀
19.	†IgG-deficiency, epilepsy, heart murmur	-	-	-	400	-	2	-	♂
20.	†IgA-deficiency, thymushypoplasia	-	-	-	400	-	2	-	♂

DPH = phenytoin; PHB = phenobarbital; PMD = primidon;
CBZ = carbamazepine; VPA = valproate; CLP = clonazepam; CLM = clobazam.
Cases nos. 9 and 13 are excluded from this analysis, since no maternal serum was obtained.
★ hydrocephalus, based on dilated ventricles at ultrasound examination
† sibs, no known immune deficiency syndrome; parents immune competent and not consanguineous, family history negative for immune deficiency.

pregnancy; or reported compliance problems (n=5). In 73 of these remaining 100 pregnancies carbamazepine was used alone. In case of polytherapy, the following combinations were used; CBZ and valproic acid (VPA) (n=8), CBZ and phenytoin (DPH) (n=2), CBZ and phenobarbital (PHB) (n=5), CBZ and clonazepam (CLP) (n=7), CBZ and clobazam (CLM) (n=1), CBZ + VPA + DPH or ethosuximide (ESX) or CLP or CLM (n=1 for each respectively).

Maternal serum. Level-dose relationships for carbamazepine and its metabolites, 10,11-epoxide and 10,11-diol in serum, all showed statistically significant linear correlations (CBZ r=0.46, 10,11-epoxide r=0.57 and 10,11-diol r=0.60). Unbound serum concentrations of carbamazepine correlated somewhat better with dose than total concentrations (CBZ r=0.54, 10,11-epoxide r=0.54, 10,11-diol r=0.62). Plots of metabolite concentrations in serum against simultaneous carbamazepine concentrations and against each other, resulted in all cases in statistically significant linear correlations

Table 2.7.2 Mean (± SD) of the daily dose per body weight ($mg.kg^{-1}.day^{-1}$) of carbamazepine and concentrations ($\mu g.ml^{-1}$) of carbamazepine, 10,11-epoxide and 10,11-diol in maternal serum and amniotic fluid.

Medication	n	Carbamazepine daily dose ($mg.kg^{-1}.day^{-1}$)	Carbamazepine concentration ($\mu g.ml^{-1}$)	10,11-epoxide concentration ($\mu g.ml^{-1}$)	10,11-diol concentration ($\mu g.ml^{-1}$)
Maternal serum					
Carbamazepine	73	9.2 ± 3.7	6.21 ± 1.84	1.18 ± 0.46	1.52 ± 0.85
+ phenobarbital	5	9.3 ± 3.7	5.53 ± 1.56	0.84 ± 0.48	0.95 ± 0.64
+ valproate	8	9.7 ± 2.3	4.91 ± 0.85	1.48 ± 0.50	1.65 ± 0.80
+ clonazepam	7	11.2 ± 3.9	6.88 ± 1.65	1.25 ± 0.46	2.19 ± 0.80
normal outcome	93	9.7 ± 3.6	6.23 ± 1.81	1.20 ± 0.46	1.57 ± 0.85
malformed	7	8.9 ± 3.7	6.24 ± 1.61	1.42 ± 0.57	1.89 ± 0.79
Unbound in serum					
Carbamazepine	73		1.58 ± 0.51	0.46 ± 0.23	1.00 ± 0.51
+ phenobarbital	5		1.32 ± 0.54	0.27 ± 0.19*	0.74 ± 0.58
+ valproate	8		1.31 ± 0.35	0.63 ± 0.28	1.16 ± 0.27
+ clonazepam	7		1.79 ± 0.46	0.65 ± 0.26	1.42 ± 0.47
normal outcome	93		1.59 ± 0.51	0.49 ± 0.25	1.04 ± 0.51
malformed	7		1.70 ± 0.52	0.62 ± 0.17	1.12 ± 0.33
Amniotic fluid					
Carbamazepine	†71		4.33 ± 1.72	1.07 ± 1.10	2.61 ± 1.47
+ phenobarbital	5		2.69 ± 1.83	0.48 ± 0.43	2.36 ± 1.01
+ valproate	8		4.18 ± 0.92	1.39 ± 0.34	2.97 ± 1.20
+ clonazepam	7		3.69 ± 1.28	0.97 ± 0.43	3.37 ± 0.81
normal outcome	93		4.13 ± 1.30	0.95 ± 0.46	2.59 ± 1.25
malformed	7		3.49 ± 1.48	0.94 ± 0.56	2.93 ± 0.69

* significantly different from all other groups ($p < 0.05$)
† "outliers" excluded (n=2)

(CBZ:10,11-epoxide r=0.52, CBZ:10,11-diol r=0.43, 10,11-epoxide:10,11-diol r=0.50)

The mean daily dose and concentrations of carbamazepine and its metabolites in maternal serum (and amniotic fluid) are shown in table 2.7.2, whereas in table 2.7.3 the data are presented as metabolite-to-parent drug ratios. Comparisons are made between different comedications as well as between normal and abnormal pregnancy outcome. Concomitant use of phenobarbital appeared to decrease plasma concentrations of carbamazepine itself, as well as both its metabolites. With the combination of carbamazepine and valproate, serum carbamazepine concentrations were the lowest, whereas 10,11-epoxide concentrations were the highest. All mothers with malformed offspring showed concentrations of carbamazepine and its metabolites within the normal variation of the group under study. Also the 10,11-epoxide to carbamazepine ratios in maternal serum were not different from the rest (table 2.7.3).

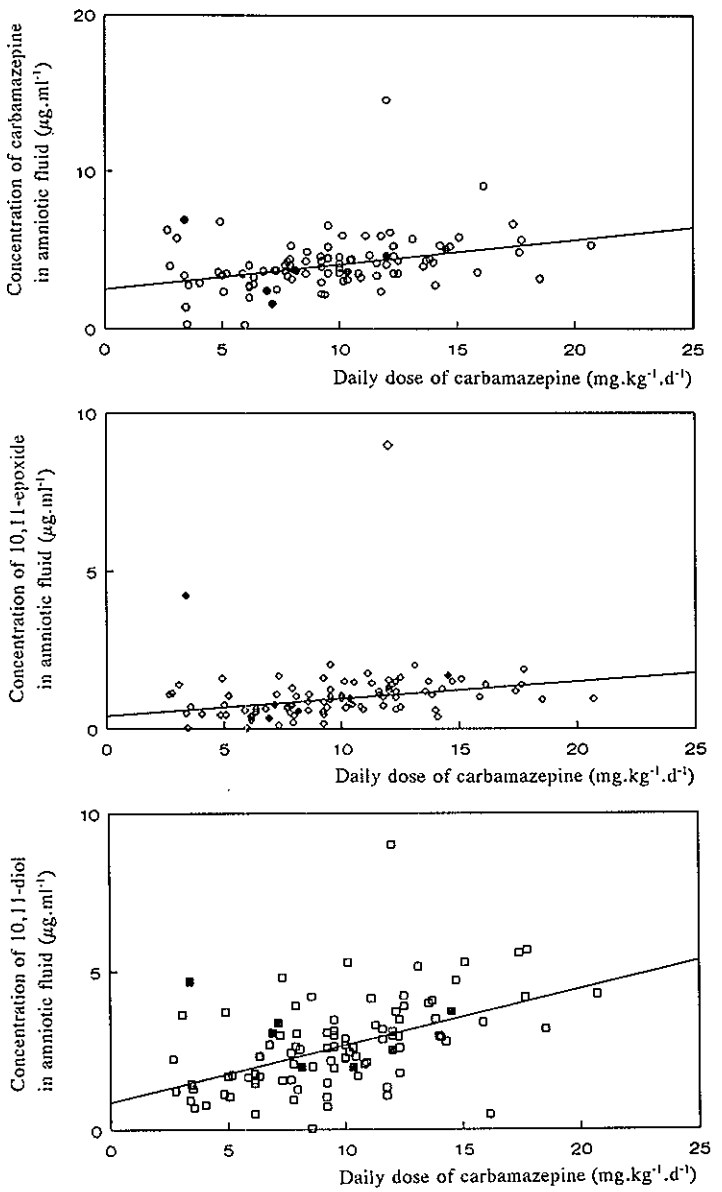
Maternal urine. The total urinary recovery through the 10,11-epoxide-10,11-diol pathway was about 40% of the administered dose. Carbamazepine excretion in urine, free or conjugated to glucuronic acid contributed little to this recovery. With valproate or clonazepam comedication, a lower percentage of the daily dose was recovered in the urine as 10,11-diol, both free and conjugated to glucuronic acid (table 2.7.4).

Table 2.7.3 Concentration ratios of metabolite-to-parent drug and metabolite-to-metabolite in maternal serum and amniotic fluid, according to comedication and pregnancy outcome

Ratios	n	CBZ dose ($mg.kg^{-1}.day^{-1}$)	10,11-epoxide/ carbamazepine	10,11-diol/ carbamazepine	10,11-diol/ 10,11-epoxide
Maternal serum					
Carbamazepine	73	9.2 ± 3.7	0.20 ± 0.08	0.24 ± 0.12	1.30 ± 0.68
+ phenobarbital	5	9.3 ± 3.7	0.14 ± 0.04	0.16 ± 0.07	1.08 ± 0.35
+ valproate	8	9.7 ± 2.3	0.31 ± 0.10*	0.36 ± 0.24	1.20 ± 0.66
+ clonazepam	7	11.2 ± 3.9	0.18 ± 0.03	0.33 ± 0.12	1.83 ± 0.69
normal outcome	93	9.7 ± 3.6	0.20 ± 0.08	1.35 ± 0.71	0.25 ± 0.12
malformed	7	8.9 ± 3.7	0.22 ± 0.09	1.40 ± 0.48	0.33 ± 0.24
Amniotic fluid					
Carbamazepine	†71		0.22 ± 0.09	0.61 ± 0.29*	3.72 ± 5.75
+ phenobarbital	5		0.15 ± 0.06	0.75 ± 0.28	4.59 ± 2.20
+ valproate	8		0.34 ± 0.07**	0.71 ± 0.22	2.18 ± 0.84
+ clonazepam	7		0.27 ± 0.12	1.04 ± 0.53	4.07 ± 2.13
normal outcome	93		0.23 ± 0.10*	0.64 ± 0.28*	3.54 ± 5.09
malformed	7		0.34 ± 0.18	0.98 ± 0.62	3.63 ± 2.75

* significantly different from other groups ($p < 0.05$).
** significantly different from group carbamazepine alone or carbamazepine + phenobarbital ($p < 0.05$).
† "outliers" excluded (n=2)

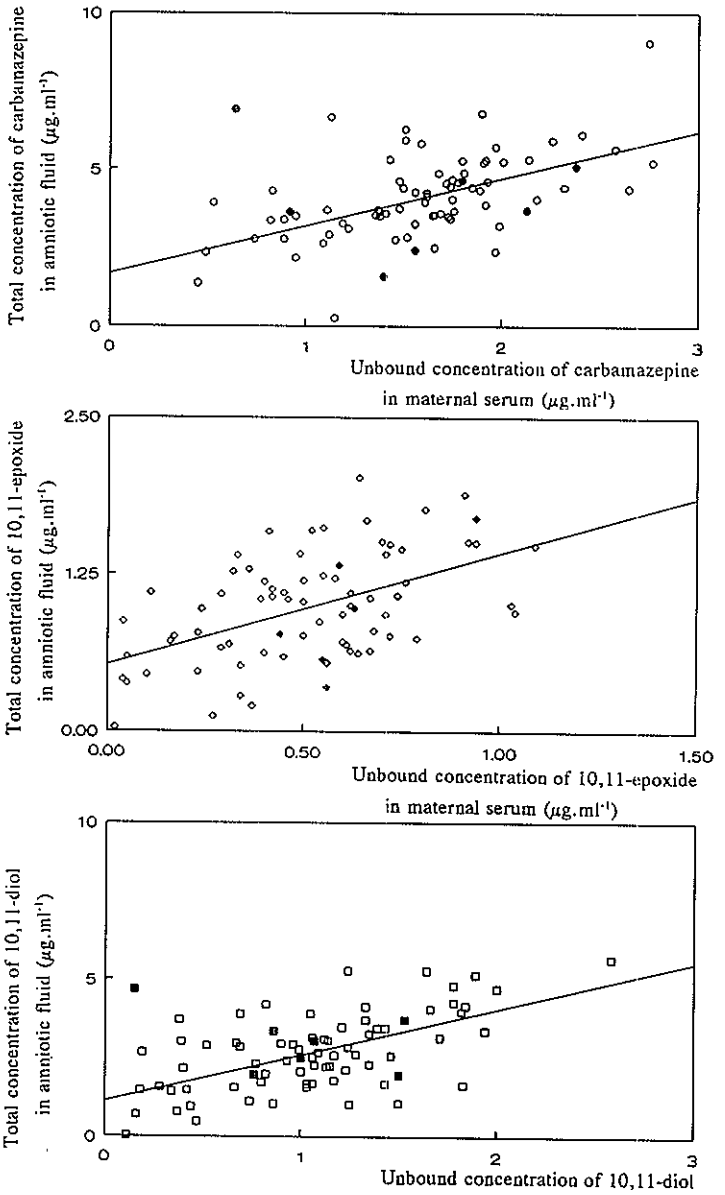
Fig. 2.7.1 Concentrations of carbamazepine (A) and its 10,11-epoxide (B) and 10,11-diol (C) in amniotic fluid *vs.* daily dose of carbamazepine



The filled symbols represent pregnancies with malformed offspring. The regression lines are described by the following equations:

for carbamazepine	y = 2.52 + 0.16x (r = 0.43)
for 10,11-epoxide	y = 0.41 + 0.06x (r = 0.45)
for 10,11-diol	y = 0.87 + 0.18x (r = 0.55)

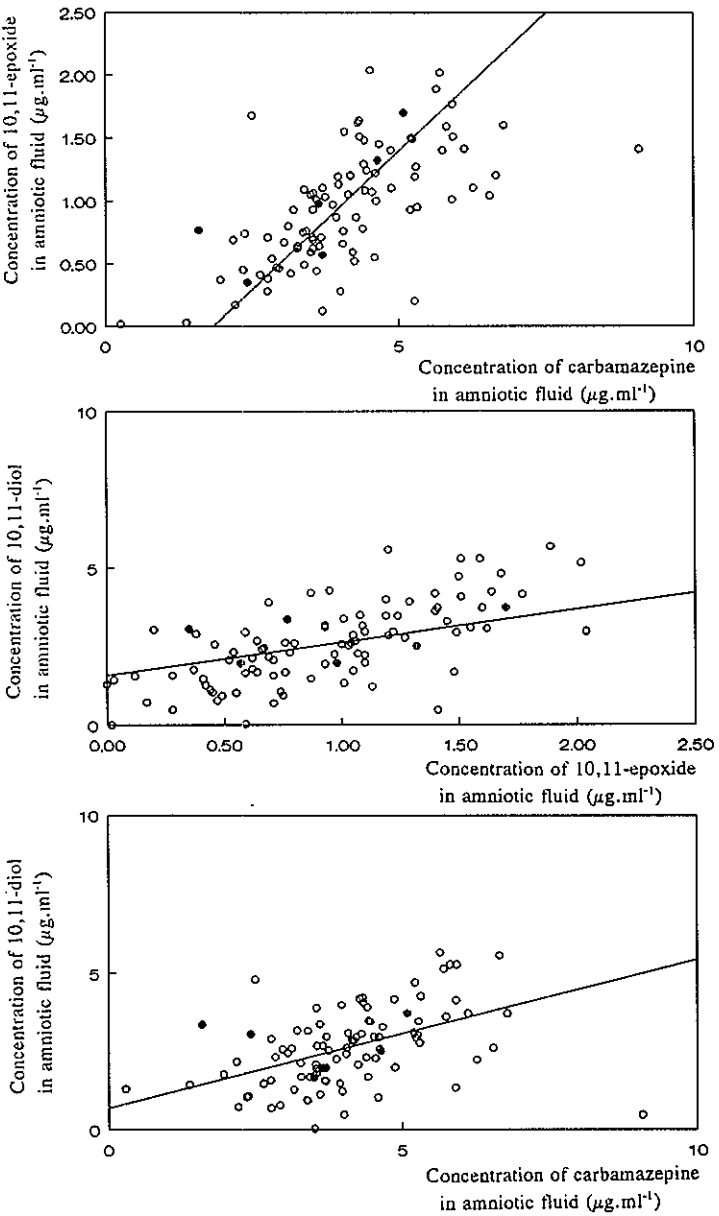
Fig. 2.7.2 Total concentrations of carbamazepine (A) and its 10,11-epoxide (B) and 10,11-diol (C) in amniotic fluid *vs.* their unbound concentration in maternal serum



The filled symbols represent pregnancies with malformed offspring. The regression lines are described by the following equations:

for carbamazepine	$y = 1.64 + 1.53x$ ($r = 0.57$)
for 10,11-epoxide	$y = 0.53 + 0.89x$ ($r = 0.51$)
for 10,11-diol	$y = 1.32 + 1.34x$ ($r = 0.56$)

Fig. 2.7.3 Relationships between concentrations of carbamazepine and its 10,11-epoxide (A) and 10,11-diol (C) as well as the relation between these two metabolites in amniotic fluid



The filled symbols represent pregnancies with malformed offspring. The regression lines are described by the following equations:
for 10,11-epoxide *vs.* carbamazepine $y = -0.81 + 0.44x$ ($r = 0.79$)
for 10,11-diol *vs.* 10,11-epoxide $y = 1.58 + 1.04x$ ($r = 0.70$)
for 10,11-diol *vs.* carbamazepine $y = 0.69 + 0.48x$ ($r = 0.57$)

Amniotic fluid. In two amniotic fluid samples very high 10,11-epoxide concentrations were found (fig. 2.7.1). One of these samples was obtained from a pregnancy which was terminated after prenatal diagnosis of a malformed fetus (case 7, CBZ 4.68 $mg.l^{-1}$, 10,11-epoxide 4.23 $mg.l^{-1}$, 10,11-diol 6.91 $mg.l^{-1}$). In the second case concentrations of carbamazepine and 10,11-diol were also exceptionally high (CBZ 8.99 $mg.l^{-1}$, 10,11-epoxide 8.98 $mg.l^{-1}$, 10,11-diol 14.60 $mg.l^{-1}$) but this pregnancy had a completely normal outcome (fig. 2.7.1). Blood contamination could be excluded. Both samples were excluded from the comparisons between different comedications.

In table 2.7.2 and 2.7.3, the data on concentrations and concentration ratios in amniotic fluid are summarized. Statistically significant linear regression lines could be fitted between total or unbound concentrations in serum *vs.* the concentration in amniotic fluid for carbamazepine and both metabolites. Amniotic fluid concentrations correlating slightly better with the unbound concentrations (fig. 2.7.2) than with total concentrations. Furthermore, concentrations in amniotic fluid of carbamazepine and its metabolites correlated strongly with one another (fig. 2.7.3). In table 2.7.5, ratios of the concentrations in amniotic fluid to total or unbound concentrations in maternal serum are presented as medians (range) since wide variations were observed.

Table 2.7.4 Mean (± SD) of the daily dose recovered as carbamazepine or through the 10,11-epoxide-diol pathway, free or conjugated to glucuronic acid, in urine.

Medication	n	Total recovery of dose (%)	Carbamazepine (%)	10,11-epoxide (%)	10,11-diol (%)
Urine					
Carbamazepine	60	43.7 ± 18.0	0.5 ± 0.7	11.8 ± 5.4	31.3 ± 12.9
+ phenobarbital	3	46.3 ± 4.0	0.2 ± 0.1	10.9 ± 2.0	35.3 ± 2.6
+ valproate	7	38.7 ± 9.2	0.3 ± 0.1	10.5 ± 3.0	27.9 ± 6.9
+ clonazepam	7	36.7 ± 10.6	0.7 ± 0.7	10.3 ± 2.2	25.7 ± 8.7
normal outcome	79	42.4 ± 16.6	0.5 ± 0.7	11.4 ± 4.9	30.5 ± 11.9
malformed	5	37.0 ± 9.7	0.7 ± 0.8	10.7 ± 2.5	25.5 ± 7.9

Not all patients contributed a 24 h urine sample.

Only one patient who was known with chronically mild hyponatraemia, had a serum sodium level below 135 mmol/l in this study. Therefore, no relationship between the occurrence of malformed offspring and hyponatraemia could be observed. No relationship between sodium concentrations and carbamazepine, or 10,11-epoxide concentrations, or 10,11-epoxide to carbamazepine ratios in serum or amniotic fluid could be found (in serum: CBZ r=-0,17, 10,11-epoxide r=-0,18, 10,11-epoxide/CBZ ratio r=-0.03, 10,11-diol/10,11-epoxide ratio r=0,10; in amniotic fluid: CBZ r=0.13, 10,11-epoxide r=0.12, 10,11-epoxide/CBZ ratio r=0.11, 10,11-diol/10,11-epoxide ratio r=0.02, in all cases $p > 0.05$).

Table 2.7.5 Mean ratios of concentrations in amniotic fluid and maternal serum

Ratio	n	Median	(range)
amniotic fluid/maternal serum (total)	98		
CBZ		0.66	(0.06 - 1.60)
CBZ-epoxide		0.75	(0.04 - 3.68)
CBZ-diol		1.74	(0.03 - 26.25)
amniotic fluid/maternal serum (free)	98		
CBZ		2.58	(0.23 - 7.43)
CBZ-epoxide		1.93	(0.06 - 21.75)
CBZ-diol		2.44	(0.36 - 14.11)

2.7.4 Discussion

Our centre covers a region with approximately 40,000 birth per year (11). With a frequency of maternal epilepsy at confinement of about 0.3% and a study period of 4.75 years, the maximum number of patients to be expected was about 570. A total of 297 pregnancies were actually included, giving an estimated participation rate of about 50%. Approximately 40% of women in the age of 20 to 40 years receiving antiepileptic drugs, are being treated with carbamazepine (12). In this study 50% of the women used carbamazepine. The clinical details of the total cohort including exposures to other antiepileptic drugs are described elswhere (13, 14).

In this cohort, the concomitant use of carbamazepine, phenobarbital and valproate, with or without phenytoin was not prescribed anymore. Although the range we observed for the 10,11-epoxide to carbamazepine concentration ratio was comparable with the study described in the introduction (2), we found some of the highest ratios with carbamazepine monotherapy. The average ratio was highest with the combination of carbamazepine and valproate and lowest with the combination carbamazepine and phenobarbital. However, neither carbamazepine or 10,11-epoxide concentrations nor 10,11-epoxide to carbamazepine concentration ratios were higher in maternal serum of pregnancies ending with malformed offspring as compared to normal outcome.

Phenobarbital reduced serum concentrations of carbamazepine, but also of its main metabolites. Thus, in addition to autoinduction the 10,11-epoxide - 10,11-diol pathway is further induced by phenobarbital. Wheras autoinduction may have a stronger effect on the activities of epoxide forming monooxygenases than on epoxide hydrolase (15), phenobarbital induces Cyt-P_{450} dependent monooxygenases as well as epoxide hydrolase (15). This is nicely confirmed by the higher percentage of the dose recovered in 24 h urine as the 10,11-diol with concomitant phenobarbital use. Valproate coadministration caused a reduction in carbamazepine concentrations and an increase in the 10,11-epoxide concentrations. Both effects are emphasized in higher 10,11-epoxide to carbamazepine ratios. The elevated 10,11-epoxide concentrations can be explained by the inhibiting effect of valproate on epoxide hydrolase, as has been demonstrated in several studies, both *in*

vitro and *in vivo* (16). This effect was also reflected in the 24 h urine data, since a slightly lower percentage of the dose was recovered through the 10,11-epoxide - 10,11-diol pathway. This might argue for an explanation why with concomitant valproate use carbamazepine concentrations are reduced. Perhaps, valproate shifts carbamazepine metabolism towards another metabolic pathway or interferes with the enterohepatic cycling of carbamazepine and metabolites. Since protein binding of carbamazepine was not decreased with valproate comedication, this could not explain the lower serum carbamazepine concentrations.

Both the mother and the fetus make individual contributions to the amniotic fluid and the extent of this varies at different stages of pregnancy. Before 12 weeks of gestation amniotic fluid is considered an extension of the fetal extracellular space (8). Around the time of amniocentesis (16 weeks) the fetus produces a small volume of urine, which is excreted into the amniotic fluid. Carbamazepine behaves as a neutral lipophilic substance and crosses the placenta with ease (17). Since it is virtually insoluble in water and is bound for 75-78% to plasma proteins one would expect it to be present in the amniotic fluid in fairly low concentrations. Indeed, the observed concentrations were on average only 70% of total concentrations in maternal serum, but up to 2 to 2.5 times the unbound concentration in maternal serum, although amniotic fluid to maternal serum ratios varied widely. Since radiolabeled maternal albumin, injected into the mother, appears initially in a higher concentration in the amniotic fluid than in the fetal blood (18), it may not be only the unbound concentration which determines concentrations in amniotic fluid. The fact that the concentrations in amniotic fluid correlate slightly better with unbound serum concentrations than with total serum concentrations, might partly be due to the lower protein concentrations present in amniotic fluid.

Concentrations of carbamazepine and metabolites in amniotic fluid correlate well with each other. Especially 10,11-epoxide concentrations in amniotic fluid correlate much better with the simultaneous concentrations of carbamazepine or 10,11-diol in amniotic fluid than with simultaneous unbound 10,11-epoxide concentrations in maternal serum. This might be expected when the amniotic cavity acts as a deep compartment, with slow equilibrium with adjacent compartments. Thereby, a small contribution from the fetus might be present, since it has been demonstrated *in vitro* that human fetal liver is able to form 10,11-epoxide (19), which in turn can be metabolized into 10,11-diol, since epoxide hydrolase is present (20).

In this study no clear relationship was found between malformed offspring and carbamazepine dose, comedication or concentrations of metabolites or parent compound in maternal serum and urine. Only metabolite to carbamazepine ratios in amniotic fluid were slightly but significantly higher in case of malformed offspring. This seems mainly due to lower carbamazepine concentrations than higher metabolite concentrations in amniotic fluid. With exclusion of the two "outliers" only the 10,11-diol to carbamazepine ratio in amniotic fluid remained significantly different.

Unfortunately, blood-samples were obtained randomly within the dosing interval, since for practical reasons blood samples could not be acquired before the morning dose. This might have introduced extra variance, since wide intradosage variation has been

described for carbamazepine (21), especially in patients on combination therapy and/or on once daily regimens. Most of our patients used carbamazepine only (73%), and most of them were on divided dosage regimens, twice or three times daily. On the assumption that most patients have taken their morning dose before visiting the prenatal care unit and the fact that in our regional centre amniocentesis is performed from 9.00 to 13.00 h, most sera have been obtained somewhere between 1 and 5 hours after administration. Besides, different patients might have used different preparations, which may result in extra variation in the time course of maximum serum concentrations (21).

If differences had been observed between normal and abnormal pregnancy outcome, with respect to daily dose and/or serum levels, as we have found in this cohort for valproate-associated spina bifida (13), than this would have been suggestive for a dose or concentration related effect, provided that systematic errors are excluded. However, we cannot preclude such an effect based on the present study, since differences in concentrations may have been masked by the random sampling procedure.

2.7.5 References

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ELECTROLYTES IN AMNIOTIC FLUID OF WOMEN ON CHRONIC ANTIEPILEPTIC DRUG TREATMENT

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Abstract

Sodium, potassium, calcium, chloride, inorganic phosphorus, uric acid, urea nitrogen, creatinine, (and total protein and albumin only in maternal serum) were determined in maternal serum and amniotic fluid obtained in midpregnancy from women on chronic antiepileptic drug treatment.
Monotherapy with carbamazepine or phenytoin was associated with significantly lower uric acid levels in maternal serum and amniotic fluid as compared to valproate and phenobarbital therapy. Pair-wise comparison of maternal serum and amniotic fluid showed that sodium, potassium, calcium and inorganic phosphorus were significantly lower in amniotic fluid, with the relatively largest difference for calcium, whereas chloride, urea nitrogen, uric acid were significantly higher.

2.8.1 Introduction

Long term treatment with antiepileptic drugs is associated with certain changes in serum electrolyte levels. Significant reductions in serum calcium may be found in many adult epileptic patients, especially when treated with enzyme inducing antiepileptic drugs (1). Besides, hyponatraemia (sodium levels < 135 mmol.l^{-1}) is a well recognized side effect of carbamazepine (2, 3). Hyponatraemia has been postulated to be a potentially teratogenic factor, based on the observation that hyponatraemia occurred in one fifth of the patients on combination therapy with carbamazepine, valproate, and phenobarbital with or without phenytoin; a combination which was also associated with a marked increased risk of congenital malformations (4). To study this further, we have evaluated electrolyte levels (sodium, potassium, chloride, calcium and inorganic phosphate) and concentrations of uric acid, urea nitrogen and creatinine in midpregnancy maternal serum and amniotic fluid.

2.8.2 Material and methods

Patients and sample collection; At the first visit (between 9 to 15 weeks of gestation, further referred to as intake) of the patient to the outpatient clinic for prenatal diagnosis informed consent was obtained. Prenatal diagnosis by transabdominal amniocentesis for α-fetoprotein determination was offered because of an increased risk for neural tube defects. Between 16 and 20 weeks of gestation, amniotic fluid was obtained from 267 women on chronic antiepileptic drug treatment (5). In 162 of these patients maternal blood was obtained just prior to amniocentesis, and only those patients are included in this part of the study. The amniotic fluid was first centrifuged for 5 min (1000 RPM) to separate cells. The supernatant was centrifuged for an additional 10 min (2000 RPM). This supernatant was used for α-fetoprotein determination as well as for clinical chemical analysis. The protocol was approved by the Medical Ethics Committee of Erasmus University Rotterdam / University Hospital Rotterdam Dijkzigt. Clinical chemical parameters were analyzed simultaneously, with a SMAC autoanalyzer according to the instructions of the manufacturer (Technicon Instruments, Garrytown, USA).

Pearson's correlation coefficients were used to determine the association between the parameters in serum and amniotic fluid. To test for differences between maternal sera and amniotic fluid Wilcoxon's matched-pairs signed ranks test was used. To search for differences in the measured parameters between various subgroups of patients on different medication the Mann-Whitney U test or Kruskal-Wallis oneway analysis of variance was used.

Table 2.8.1 Comparison of some clinical chemical parameters between groups of pregnant women on different antiepileptic drug monotherapies

Parameter		Carbamazepine n=65	Valproate n=42	Phenytoin n=13	Phenobarbital n=10	p-value
Maternal serum, obtained prior to amniocentesis (gestational age 17 ± 1 weeks)						
Sodium	(mmol.l^{-1})	141 ± 2	142 ± 2	141 ± 2	142 ± 2	0.18
Potassium	(mmol.l^{-1})	4.4 ± 0.6	4.4 ± 0.5	4.6 ± 1.2	4.3 ± 0.2	0.87
Calcium	(mmol.l^{-1})	2.29 ± 0.09	2.31 ± 0.06	2.27 ± 0.08	2.30 ± 0.07	0.30
Phosphorus	(mmol.l^{-1})	1.10 ± 0.18	1.07 ± 0.18	1.18 ± 0.17	1.10 ± 0.04	0.24
Chloride	(mmol.l^{-1})	105 ± 2	106 ± 2	106 ± 2	106 ± 2	0.51
Urea nitrogen	(mmol.l^{-1})	3.2 ± 0.8	3.2 ± 1.1	2.9 ± 0.8	3.2 ± 1.0	0.73
Uric acid	(mmol.l^{-1})	0.16 ± 0.03	0.20 ± 0.04	0.15 ± 0.04	0.19 ± 0.04	<0.0001
Creatinine	(μmol.l^{-1})	51 ± 5	51 ± 5	50 ± 5	52 ± 6	0.91
Total protein	(gr.l^{-1})	65 ± 3	64 ± 3	65 ± 4	67 ± 4	0.43
Albumine	(gr.l^{-1})	40 ± 2	40 ± 2	40 ± 3	41 ± 2	0.69
Amniotic fluid (gestational age 17 ± 1 weeks)						
Sodium	(mmol.l^{-1})	137 ± 6	136 ± 9	137 ± 4	139 ± 2	0.36
Potassium	(mmol.l^{-1})	4.2 ± 0.2	4.1 ± 0.3	4.2 ± 0.1	4.2 ± 0.2	0.34
Calcium	(mmol.l^{-1})	1.41 ± 0.37	1.49 ± 0.24	1.51 ± 0.25	1.40 ± 0.24	0.67
Phosphorus	(mmol.l^{-1})	0.96 ± 0.20	0.95 ± 0.17	0.93 ± 0.16	0.94 ± 0.14	0.96
Chloride	(mmol.l^{-1})	112 ± 5	111 ± 9	112 ± 4	114 ± 2	0.44
Urea nitrogen	(mmol.l^{-1})	3.8 ± 0.9	3.8 ± 1.3	3.9 ± 0.9	4.1 ± 1.2	0.88
Uric acid	(mmol.l^{-1})	0.19 ± 0.04	0.24 ± 0.04	0.19 ± 0.05	0.23 ± 0.05	<0.0001
Creatinine	(μmol.l^{-1})	53 ± 12	52 ± 13	49 ± 12	57 ± 10	0.48

Table 2.7.2 Summary of data on clinical chemical parameters in amniotic fluid and maternal serum, obtained prior to amniocentesis (gestational age 17 ± 1 weeks)

Parameter		Maternal serum (MS)	Amniotic fluid (AF)	Mean difference (MS-AF) n=162	p-value
Sodium	(mmol.l^{-1})	141 ± 2	137 ± 6	4 ± 6	<0.001
Potassium	(mmol.l^{-1})	4.4 ± 0.6	4.2 ± 0.2	0.2 ± 0.6	<0.001
Calcium	(mmol.l^{-1})	2.29± 0.08	1.44 ± 0.30	0.85± 0.29	<0.001
Phosphorus	(mmol.l^{-1})	1.10± 0.18	0.95 ± 0.17	0.15± 0.22	<0.001
Chloride	(mmol.l^{-1})	105 ± 2	112 ± 3	-7 ± 5	<0.001
Urea nitrogen	(mmol.l^{-1})	3.2 ± 0.9	3.8 ± 1.0	-0.6 ± 0.6	<0.001
Uric acid	(mmol.l^{-1})	0.18± 0.04	0.21 ± 0.05	-0.03± 0.03	<0.001
Creatinine	(μmol.l^{-1})	51 ± 6	53 ±12	-1 ± 12	<0.224
Total protein	(gr.l^{-1})	65 ± 3	nd	-	
Albumine	(gr.l^{-1})	40 ± 2	nd	-	

nd = not determined

2.8.3 Results

With regard to individual patients, only one patient on carbamazepine monotherapy had serum sodium levels slightly below 135 mmol.l^{-1}, the lower level of normal. She was known to her neurologist to have chronically mild hyponatraemia. This pregnancy ended with the birth of a healthy child. No other clinically important deviations from the normal range in serum were observed in any of the patients. Of the 162 pregnancies, included in this part of the study, 15 ended with the birth of a malformed fetus or infant. The abnormalities observed were: 6 fetuses with spina bifida aperta (including 1 pair of twins), 2 club foots, an inguinal hernia, hypospadia with ptosis, hypertrophic pylorusstenosis, cleft lip, congenital heart defect in combination with hemivertebrae and left radiusaplasia, two sibs with immune deficiencies and a child with microcephaly (for details see ref 5). In none of these 15 pregnancies abnormalities in any of the measured parameters could be demonstrated in maternal serum or amniotic fluid.

Comparison of patients on different monotherapies (carbamazepine (CBZ), valproate (VPA), phenytoin (DPH) and phenobarbital (PHB)) revealed statistically significant lower concentrations of uric acid in maternal serum as well as in amniotic fluid with carbamazepine or phenytoin treatment (table 2.8.1). With carbamazepine monotherapy serum sodium was also significantly lower as compared with all three other subgroups (p=0.008). This difference was not observed in amniotic fluid. No other significant differences were observed between the four subgroups. With phenytoin monotherapy serum uric acid as well as serum calcium, urea nitrogen and creatinine were slightly lower than in the other subgroups. When monotherapy was compared with polytherapy, the effect on the reduction of uric acid serum levels could also be

demonstrated when carbamazepine or phenytoin were administered concomitantly with valproate (VPA alone 0.20 ± 0.04 mmol.l^{-1} (n=42) vs. VPA with CBZ or DPH 0.16 ± 0.02 mmol.l^{-1} (n=13) *vs.* 0.19 ± 0.05 mmol.l^{-1} (n=6) for VPA with PHB or other antiepileptic drugs, p=0.006).

Table 2.8.2 summarizes the data for amniotic fluid (AF) and maternal serum (MS), obtained just prior to amniocentesis, giving means (M) and standard deviations and mean differences (MS-AF). Pair-wise comparison between maternal serum and amniotic fluid demonstrated that all measured parameters, except creatinine, were significantly different in both liquids. Whereas sodium, potassium, calcium and inorganic phosphorus were higher in maternal serum than in amniotic fluid, the opposite was true for chloride, urea nitrogen and uric acid (table 2.8.2).

2.8.4 Discussion

For the group as a whole, no clinically relevant deviations were observed in maternal serum as compared to non-pregnant reference values used in our clinic, except for serum creatinine which was well below the lower level of the reference range of non-pregnant individuals. However, this is a normal physiological change seen in pregnancy, due to changes in renal blood flow and glomerular function, resulting in increased filtration with a fall in blood creatinine. This effect is maximal in midpregnancy (6). Often urea nitrogen and uric acid are also reduced, but those parameters remained within the reference range in this study.

In this cohort, slightly reduced calcium levels where found. Long term treatment with phenytoin, phenobarbital or carbamazepine is associated with reduced calcium levels (1, 7, 8, 9). This effect on calcium metabolism is thought to be due to the inducing effect of these drugs on liver enzymes, causing a disturbance in the hydroxylation of vitamin D_3 to 25-OH-cholecalciferol, which is the immediate precursor of 1,25-dihydroxycholecalciferol, the active form of vitamin D on bone and intestine. Besides an inducing effect, increased excretion of hydroxylated vitamin D metabolites is also suggested (7, 8).

Two cases of neonatal hypocalcemia after intrauterine exposure to a combination of phenobarbital and phenytoin have been reported in the literature by Friis and Sardemann (10). They assumed that the interference with vitamin D metabolism during intra-uterine life could be responsible for defective fetal bone mineralisation. Within this cohort one premature infant exposed in utero to carbamazepine (800 mg.d^{-1}) showed hypocalcemia at birth, which was most likely related to prematurity. Calcium concentrations in maternal serum and amniotic fluid in this pregnancy were not reduced.

Comparison of different monotherapies revealed that uric acid was significantly reduced with carbamazepine and phenytoin treatment compared to phenobarbital and valproate treatment. This phenomenon has been described in the literature (11) and several speculations on the mechanism involved have been made. Krauss suggested that acceleration of protein synthesis, caused by the enzyme-inducing properties of some

anticonvulsants, may lead to lower uric acid serum levels (11). This is, however, not in agreement with the enzyme inducing activity of phenobarbital with which uric acid levels are not reduced. Although the different anticonvulsants might exert induction to a different degree, it is unlikely that this would lead to this difference.

Hypouricaemia has been shown to occur in hyponatraemia due to the syndrome of inappropriate antidiuretic hormone secretion (SIADH) and this observation (12) has been used as a simple means to distinguish SIADH from other causes of hyponatraemia. Normally, more than 90% of excreted uric acid is reabsorbed to maintain high serum levels. The low serum uric acid levels in SIADH appear to be due to an increase in fractional urinary excretion of uric acid. This effect is secondary to volume expansion (13). The antidiuretic effect of carbamazepine and the observed correlation with reduced sodium and urate levels in patients on carbamazepine monotherapy also suggest an interference with antidiuretic hormone. Whereas some authors believe that the reduction in serum sodium associated with carbamazepine treatment may be related to inappropriate secretion of antidiuretic hormone (14), others have suggested that the drug or some of its metabolites might exert an antidiuretic hormone-like effect on renal tubulus (4). Data are conflicting in this regard (4) and the precise mechanism is not fully understood.

An interesting hypothesis with regard to hypouricaemia is one postulated by Ames and colleagues, several years ago (15). They demonstrated that uric acid is a powerful antioxidant and a scavenger of reactive oxygen species. Since plasma urate levels in human are considerably higher than ascorbate levels, they postulated that it is one of the major water soluble antioxidants in humans. This postulated protective mechanism against oxidative stress might be negatively influenced, when carbamazepine (and phenytoin) changes renal urate handling. Alternatively, reduced uric acid serum levels after chronic treatment with carbamazepine and phenytoin may also be a sign of depletion of the uric acid pool, as a result of the presence of potentially reactive metabolites.

The regulation of volume and composition of amniotic fluid is still not completely understood. In general it is thought that in early pregnancy amniotic fluid is an extension of fetal extracellular fluid space, since the fetal skin is permeable for water and some solutes during this stage. With proceeding pregnancy, the fetal skin becomes keratinized and impermeable, and the composition of amniotic fluid deviates progressively from any fluid compartment in mother or fetus. During the second and third trimester, exchanges of water and solutes between the fetus and the amniotic fluid take place by fetal swallowing and reabsorption by its intestine, via the respiratory tract and by fetal micturation. Exchange may also occur across the chorionic plate between the fetal circulation and the amniotic fluid (17).

Our data in amniotic fluid are in general agreement with the literature (16, 17, 18). Concentrations of sodium and potassium were lower in amniotic fluid than in maternal serum, whereas chloride levels were somewhat higher. Total calcium concentrations in amniotic fluid are approximately 63% of those in maternal serum. This relatively large difference can partly be explained by the relationship between total calcium and total protein, since amniotic fluid contains considerably less protein than serum does. A small decrease in electrolyte content and increase in concentrations of uric

acid and creatinine in amniotic fluid might be explained by the addition of hypotonic fetal urine. From the end of the first trimester onwards small amounts of fetal urine, which has a low sodium and a relatively high urea content (17), may be influencing the final concentrations in amniotic fluid.

2.8.5 References

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CHAPTER 3

IMPLICATIONS FOR MEDICAL CARE

RECOMMENDATIONS FOR THE CARE OF EPILEPTIC WOMEN OF CHILDBEARING AGE AND PERSPECTIVES FOR FUTURE RESEARCH

3.1 Implications for counselling of epileptic women

Consensus Guidelines for the care of epileptic women of childbearing age are developed by the Commission on Genetics, Pregnancy, and the Child of the International League Against Epilepsy (ILAE). These guidelines published in 1989 (1) are still valid and will be discussed below, with addition of some experiences of our study population.

Risk of major malformations

Future epileptic parents should be informed of the possibility of congenital anomalies in their children, preferably before conception in order to allow adjustment of therapy. In this respect it has to be reminded that enzyme-inducing antiepileptic drugs, like phenobarbital, phenytoin and carbamazepine, might all decrease the reliability of oral contraceptives, and appropriate measures should be taken (2).
It should be told that the offspring of epileptic women with first trimester antiepileptic drug exposure have a 2 to 3 fold increase in risk of birth defects, as compared to the general population. For specific malformations the risk might be considerably higher than 2 to 3 fold. For cleft lip and/or palate the estimated risk is about 10 fold ($\approx$1%) and for congenital heart anomalies 4 fold ($\approx$2%), with an apparent drug specificity towards phenytoin and phenobarbital. The risk for spina bifida is about 20 fold ($\approx$1-2%) and specifically associated with valproate exposure. For carbamazepine the risk for spina bifida appears to be increased about 10 fold ($\approx$1%) as compared to the general population, but this requires further evidence.

It should be emphasized that more than 90% of the women with epilepsy receiving antiepileptic drugs will deliver a healthy infant.

Adjustment of drug therapy and changes in seizure frequency?

There is general agreement that most epileptic women continue to require treatment during pregnancy, to minimize the risk of trauma for the woman herself as well as the fetus. The dangers of abrupt withdrawal of antiepileptic drug therapy, *i.e.* recurrence or increase in seizure frequency or even status epilepticus, as well as the possible adverse effects of such increase in seizure frequency on the fetus, must also be discussed.

Since, we are not able to predict which of the 4 most frequently used antiepileptic drugs (phenytoin, phenobarbital, carbamazepine, and valproate) bear the lowest risk of teratogenicity for a particular patient, the woman should be treated according to the usual therapeutic rules, *i.e.* the drug of first-choice to treat the seizure type or epilepsy should be used as monotherapy at the lowest effective dose. With substitution of monotherapy for polytherapy and decreasing the dose as much as possible, it has been possible to significantly reduce the incidence of malformations (3, 4). With respect to the 1-2% risk of spina bifida with first trimester valproate exposure, a possible replacement of this drug, or dose reduction as well as dividing the doses over 3 to 4 administrations per day to avoid high plasma concentrations, must be carefully considered (paragraph 2.2).

In the literature, no increased risk for the offspring of unexposed epileptic women compared to non-epileptic women was evident, although numbers of unexposed epileptic women are limited. Nevertheless, this would favour the approach to discontinue antiepileptic drugs in those women with child wish, who are seizure free for several years.

Since no control study has been performed on the effects of antiepileptic drug withdrawal in women planning pregnancy or during pregnancy, we are depending on the results of withdrawal in the epileptic population at large (5-7). However, these studies showed that seizure relapse after withdrawal varied widely in patients who had been completely seizure free for two to five years on antiepileptic drugs treatment. It appeared that relapse frequency was higher in case of a history of generalized tonic-clonic seizures or myoclonias or awakening seizures (7). Also, patients who maintained only seizure free on a combination of two or more antiepileptic drugs had a higher risk of relapse of seizures (5-7). In case of seizure recurrence or increase in frequency, treatment should be restarted. When one is informed that the patient on medication is already a few weeks pregnant, there is probably no point in changing the anticonvulsant medication.

As mentioned above therapy changes should be considered before conception with slow withdrawal over one to three months, under close monitoring. However, to further complicate the picture, pregnancy can have an unpredictable effect on seizure frequency, both from patient to patient as well as during different pregnancies in the same woman. Several former studies have shown that in about one-quarter of women seizure frequency increases during pregnancy, in another quarter seizure frequency decreases and in the remaining half of the group no significant change occurs (8). Although, in more recent studies an increase in seizure frequency was more probable than a decrease (9, 10), this could be confounded by more intended non-compliance because of fear for harming the

fetus, caused by the increasing amount of information on this topic. Any attempt to identify possible risk factors for increased seizure frequency during pregnancy has failed up to now. In this regard several features of the underlying epilepsy have been investigated, besides possible endocrine and metabolic changes induced by pregnancy including modifications of antiepileptic drug disposition or pregnancy related psychological problems, including noncompliance and sleep deprivation, none of these factors appears to be of prognostic value in predicting the clinical course of epilepsy (9, 10).

Modification of antiepileptic drug disposition has been proposed as the main reason for increased seizure frequency. This is mainly expressed as a fall in plasma concentrations as pregnancy proceeds and a subsequent increase during the puerperium. The extent of these changes might differ between different antiepileptic drugs, and appears to be greatest for phenytoin, less with phenobarbital and not significant with primidone, carbamazepine and valproate (12). With the exclusion of non-compliance, causes for this fall in plasma concentrations might be related to reduced absorption due to gastrointestinal changes inherent to pregnancy, such as decreased bowel motility, leading to a lower rate of intestinal absorption, or modifications of gastric juice, leading to alterations in bioavailability. Changes in the apparent volume of distribution like maternal body weight gain and increased water content, decreased plasma protein binding due to the gradual decrease of plasma proteins during pregnancy, or changes in levels of endogenous binding inhibitors might also be of influence. Finally, an increase in the hepatic metabolism or renal excretion of certain antiepileptic drugs during pregnancy might also contribute to these changes (13).

When low plasma concentrations are monitored, certainty about compliance has to be obtained. Dose adjustment should only take place when seizures occur. If the dose has been increased during pregnancy, blood levels should be continued to be monitored up to the puerperium, with appropriate dose reduction in this period.

Periconceptional folic acid supplementation?

Although results from the multivitamin study group with regard to folic acid suppletion prior to conception and during the first three months of pregnancy has been shown to reduce the recurrence risk for neural tube defects considerably, the value of folate supplementation in improving pregnancy outcome in antiepileptic patients receiving antiepileptic treatment in general, and the prevention of neural tube defects associated with valproate or carbamazepine exposure more specifically, remains unsolved (paragraph 1.2). The general consensus up to date with regard to folic acid supplementation is to treat possibly existing folic acid deficiencies, whereas for patients with normal folic acid red blood cell levels an appropriate diet before conception and during pregnancy should be sufficient (14). This is in accordance with recent guidelines from the Dutch State Supervision of Public Health (15).

Pre-pregnancy counselling should include the above mentioned possibilities and possible consequences of discontinuation of antiepileptic drugs. It should be emphasized that a possible withdrawal should be closely monitored and therapy should be restarted in case of relapses of seizures. If the woman experiences tonic-clonic or frequent partial seizures withdrawal is no option. Intended non-compliance, due to fear of fetal damage should be discouraged. It should be explained that organogenesis takes place within the first eight weeks after conception. The possibility of seizures induced by abrupt withdrawal or non-compliance would probably not prevent the occurrence of these early induced structural malformations but would put herself and the fetus in jeopardy by increased seizure risk.

It should be emphasized that general guidelines like a good general health with adequate sleep, good nutrition and the discouraging of the consumption of cigarettes, alcohol and drugs, others than prescribed by a physician as well as regular neurological follow-up for seizure control could all contribute to a good pregnancy outcome. Although a slight increase (1.5 to 3 times) in common pregnancy complications, such as toxaemia, preeclampsia, placental abruption, as well as more premature labour or labour interventions is described to occur more often in women with epilepsy, these data are mainly based on retrospective and register-based studies and are not general agreed upon (16).

Since the possibility exists that some of the risk is caused by a genetic predisposition for birth defects inherent in certain families, both parents' family histories should be reviewed for birth defects. In case a patient treated with valproate or carbamazepine has a positive family history of neural tube defects and is not yet pregnant, a therapy change to phenytoin or phenobarbital has been advised by some authors (15). Furthermore, a contribution of predisposing genetic factors in case of valproate-induced spina bifida is expected, since of the 34 infants described in paragraph 2.3, four cases consisted of two pairs of siblings whose mothers were exposed to valproate during consecutive pregnancies, and two cases of a monozygotic pair of twins, concordant for the defect.

The possibility of prenatal diagnosis of certain birth defects should be offered and the attitude of the parents toward these procedures and selective abortion should be discussed. In case of unavoidable valproate or carbamazepine treatment prenatal diagnosis of neural tube defects with amniotic fluid analysis of α-fetoprotein at 16 weeks of gestation in combination with ultrasonography should be offered. Although some disagreement (16, 17) exists about whether amniocentesis should be offered directly or whether it should be limited to those cases where high resolution ultrasound examination and elevated maternal serum α-fetoprotein levels fail to exclude a neural tube defect, in The Netherlands direct amniotic fluid determinations are preferred (18), since this limits the period and experience of uncertainty, and often the diagnosis is obtained earlier in gestation. Furthermore, in our series only two of the five affected pregnancies showed multiples of the median above the cut-off level for maternal serum α-fetoprotein. Serum

α-fetoprotein as a screening method might not be valid for a high risk population like valproate or carbamazepine exposed fetuses (paragraph 2.4).

High-resolution ultrasonography performed by an experienced examiner can detect more than 94% of neural tube defects. The position of the fetus or placenta or maternal obesity might preclude proper visualisation. Since weight gain might be a side effect of the drug, maternal obesity might be a complication in some women on valproate therapy. Only two of the neural tube defects in this series were first encountered during ultrasound examination, albeit the others were confirmed. With our approach we can not preclude that ultrasound examination at a later stage in pregnancy would have been able to detect the other cases. Some authors estimate that only 1% of the neural tube defect cases would go unnoticed when both serum α-fetoprotein levels and ultrasound scan are normal (16, 17) and this should be weighed against the 0.5-1% risk for miscarriage associated with amniocentesis. A variety of other major malformations can also be diagnosed by high resolution ultra-sonography during the 18th to 22nd weeks of gestation, but it should be emphasized that only certain structural defects can be visualized, like orofacial clefts and heart defects, and not in all cases (19).

The enlightenment of the possibility of prenatal diagnosis after antiepileptic drug use has led to an estimated participation rate in our clinic of about 50%, which is in the same order of magnitude as on the indication maternal age above 36 years (20).

After selective abortion based on the result of prenatal diagnosis the need of autopsy to document the malformations is self evident. In addition, Blumberg was the first to stress the need for supportive counselling both before and after selective abortion (21). In case of women with epilepsy the possibility of seizures should be considered again. Emotional and physical stress, as well as possibly deliberate non-compliance due to an understandable aversion against the "putative" drug, might all provoke seizures. Seizures might also occur during the termination itself. Of the five pregnancies terminated in this study group, three out of four women had to experience seizures during termination conducted with prostaglandins. These results have led to the decision to change termination procedures by using hypertone salt (22). However, in only one of these pregnancies drug levels were monitored and non-compliance was proven. Therefore, without drug monitoring it is preliminary to accuse prostaglandins and more important, maximum care and information to the patient should be given in order to maintain appropriate medication.

Supportive counselling after a selective abortion, should also include post-terminations sequelae, to prevent confusion and bewilderness by the post-partum reactions of the woman's body (*i.e.* lactation and mastitis) and the strong emotions. During a post-termination appointment the parents also have an additional possibility to ask questions or to discuss the fetal abnormalities, obtain photographs if requested and to be counselled about the risks in subsequent pregnancies.

Genetic counselling in case of maternal epilepsy covers, besides the risk of possible teratogenic effects of antiepileptic drug treatment, the risk of seizures during pregnancy and the influence of pregnancy on seizures, also the possible genetic aspects for the child to develop epilepsy, but this subject goes beyond the scope of this thesis and

besides the available data is still limited. Epilepsy is a collection of functional disorders of the central nervous system with various causes and clinical expressions. Up to date, in most cases, only crude recurrence risk estimates and vague descriptions about the possible phenotypic appearance can be given, since genetic (and phenotypic) heterogeneity and irregular patterns of inheritance complicate the picture. Only for a minority of specific types of epilepsy, Mendelian inheritance has been established and a clear-cut recurrence risk estimate can be offered (23). Through molecular genetic studies some progress might be expected in the near future.

3.2 Future research

The role of antiepileptic drugs in the increased risk of birth defects among offspring of epileptic mothers remains poorly understood, due to difficulties inherent to studying teratogenicity in general.

Further human studies should focus on quantitative comparisons of congenital anomaly risk associated with specific antiepileptic drug therapy. Since, the clinically important question remains which treatment regimens have the lowest teratogenic risk. Definite conclusions can not be drawn from individual investigations, due to small numbers and methodology might be inadequate. Therefore, besides birth registries, large (at least 1600 pregnancies) international prospective studies with clinical data on precise exposure, epilepsy and inheritance remain essential, partly to obtain sufficient numbers of specific therapies to establish the therapy with the lowest risk and to search for factors which could help to predict adverse outcome. Such a comprehensive protocol has been established by the ILAE, commission on Pregnancy, Epilepsy and the Child. Evaluation of the teratogenic potential of newly introduced antiepileptic drugs in an earlier phase giving full consideration to possible confounding factors would also be possible with this approach. Thereby, postnatal intellectual development should get more attention. Within these prospective studies, the frequency of spina bifida occulta in infants exposed to valproate *in utero* compared with other antiepileptic drug exposure as well as the general population could also be estimated.

Further insight in the effects of folic acid supplementation in pregnancies with antiepileptic drug use might be impossible to study. Firstly, because numbers of exposed pregnancies as well as a possible reduction in malformation frequency will not be sufficient to observe statistically significant differences. Secondly, some people believe that ethical considerations might already limit randomized studies concerning folic acid supplementation. Therefore, data on this subject will probably be restricted to patients in future prospective studies who inform that they do or do not receive folic acid supplementation and determination of folic acid status (preferably red cell folate levels) around the time of conception should be included if possible.

More insight in the genetic background of epilepsy, as well as the etiology of specific birth defects is needed before more precise advice regarding antiepileptic treatment can be given. Investigations into the possible mechanisms of the potential

teratogenic effects of antiepileptic drugs could in the end help to improve risk assessment. The search for genetic variation in drug metabolism as a possible basis for genetic predisposition might best be continued by a panel approach, using different probe drugs. A proper probe drug for pharmacogenetic studies will be metabolized mainly through the metabolic route under study. Both valproate and carbamazepine are metabolized through many different pathways which interconnect with each other or even as for valproate with intermediary metabolism. Moreover, several of these routes might be induced to different extents by several agents and all this limits the detection of possible differences. Family studies and *in vitro* biological assays using patients cells or cell lines, or genotyping by analysis of genes encoding for (the regulation of) enzymes involved in drug metabolism, may further contribute to elucidate which genetic factors predispose to environmentally induced malformations. Eventually, elucidation of predisposition *before* exposure would improve risk assessment before pregnancy and would allow preventive adjustment of therapy (24).

3.3 References

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SUMMARY

There is strong consistent evidence that the offspring of epileptic women with first trimester antiepileptic drug exposure have a two to three fold increase in risk of congenital malformations as compared to non-epileptic women. Moreover, this risk appears higher as compared to untreated mothers and fathers with epilepsy. This suggests an etiologic role for antiepileptic drugs in the induction of the observed congenital anomalies. However, this remains a controversial issue since it is very difficult to distinguish between the treatment and the underlying illness and seldom a specific malformation is observed with one particular drug. The literature on these issues, as well as some postulated mechanisms through which antiepileptic drugs might exert teratogenic effects are discussed in chapter 1.

Neural tube-defects appear to be linked specifically to the use of valproate (estimated risk 1-2%) and carbamazepine (estimated risk 0.9%). Since this risk is 10 to 20 folds higher than the risk in the general population (0.05-0.1%) and similar to the risk for parents with a previous child born with a neural tube defect, use of these above-mentioned drugs during the first trimester of pregnancy has become an indication to offer amniocentesis. A short overview on the regulation of amniotic fluid and the distribution of drugs in it is given in section 1.3.

In cooperation with the outpatient clinic for Prenatal Diagnosis and the Laboratory for Prenatal Diagnosis of the University Hospital Rotterdam - Dijkzigt, a prospective cohort study was conducted from November 1985 until July 1990 to further investigate the precise causative relation between anti-epileptic drug use during pregnancy and the occurrence of neural tube defects (§ 2.1).

Evaluated were 297 pregnancies of 261 different epileptic women referred for prenatal diagnosis in connection with chronic antiepileptic medication. In 5 of the 92 pregnancies (5.4%) with maternal valproate use, spina bifida was prenatally diagnosed; no cases of anencephaly were observed. The occurrence of spina bifida was associated with a significantly higher average daily dose of valproate (500 mg per day more) as compared to pregnancies with normal outcome. There was no relation with the type of epilepsy, medical history, or family history of epilepsy or neural tube defects. When valproate medication cannot be avoided, the teratogenic risk may be diminished by reduction of the daily dose and of the dose per administration (§ 2.2).

Among the 34 neural tube defect cases known to us from different sources within The Netherlands, spina bifida was also observed far more frequently than anencephaly (33:1), suggesting a specific association with caudal defects. Most neural-tube defects following

maternal valproate use are severe open defects, often associated with hydrocephaly and other midline defects, like hypospadias (2), hypertelorism (2), ventricular septal defect, and several other intracerebral deformities were observed more often (§ 2.3).

All five spinal defects associated with valproate use, observed in our prospective cohort, were prenatally diagnosed by α-fetoprotein determination in amniotic fluid and confirmed by acetylcholinesterase electrophoresis. In only two of these five affected pregnancies maternal serum Ó-fetoprotein levels were raised. Isolated maternal serum α-fetoprotein levels may be unreliable for prenatal diagnosis of neural tube defects in women taking valproate (§ 2.4).

Continuation of antiepileptic drug treatment during pregnancy is often a necessity. Research should be directed to which treatment regimens have the lowest teratogenic risk. Determination of other possible risk-increasing factors would help to improve counselling.

In this cohort, 76% of pregnancies were treated with only one antiepileptic drug. Carbamazepine was the most frequently used drug (54%), either as monotherapy (38%) or polytherapy (16%). Of the 303 newborns, 6.9% had a congenital malformation, among which spina bifida (6), congenital heart defects (n=2), hypospadias (n=2), and facial clefts (n=3). After exclusion of valproate-associated spina bifida cases, partial epilepsy of the mother, and a positive family history of epilepsy, also on the paternal side appear to be associated with an increased risk of congenital malformations. Previous malformed offspring also appeared to be associated with an increased risk. Whether this reflects a genetic susceptibility to certain malformations or a predisposition for teratogenic effects with similar exposures during successive pregnancies remains subject for further study. None of the antiepileptic drugs as such showed a significantly higher risk as compared with all others (§ 2.5).

To test the hypothesis if differences in metabolism of valproate and carbamazepine could explain differences between individuals in the occurrence of congenital malformations, concentrations of above-mentioned drugs and several of their metabolites were determined in amniotic fluid, maternal serum and 24 hours urine samples.

In 52 pregnancies of women taking long-term valproate we have measured the concentrations of the parent compound and 13 of its metabolites by gas-chromatography-mass spectrometry. Concentrations in amniotic fluid of valproate and several of its metabolites ((E)Δ^2-valproate, (2E,3'E)$\Delta^{2,3'}$-valproate and 3-keto-valproate) correlated with total concentrations of valproate as well as with unbound concentrations of valproate in maternal serum. We suggest that the amniotic fluid acts as a deep compartment. The data further suggest that during the first and early second trimester of pregnancy the ß-oxidation of valproate decreases. In pregnancies with fetal neural tube defects significantly higher daily doses of valproate were used, resulting in higher concentrations of valproate in maternal serum. The metabolite patterns in maternal serum, 24 h urine samples, and amniotic fluid did not show any significant differences. These results further support the hypothesis that valproate itself and not one of the measured metabolites is the ultimate teratogen (§ 2.6).

Concerning carbamazepine use, especially in polytherapy, epoxide metabolites have been suggested to play a role in the occurrence of congenital malformations. We have investigated the 10,11-epoxide - 10,11-diol pathway of carbamazepine in 100 pregnancies (including 7 with malformed outcome) with first trimester carbamazepine exposure, alone or

in combination with other antiepileptic drugs. In maternal serum, carbamazepine-10,11-epoxide concentrations increased with increasing dose. Comedication with phenobarbital results in lower 10-11-epoxide concentrations, with a higher percentage of the dose recovered in the urine as 10,11-diol. Polytherapy with valproate results in higher 10,11-epoxide concentrations in combination with slightly lower concentrations of carbamazepine. With valproate as well as clonazepam comedication a lower percentage of the dose was recovered in the urine as 10,11-diol. In amniotic fluid, concentrations of carbamazepine and its main metabolites were in most patients 2 to 2.5 times higher than the unbound concentrations in maternal serum. In this prospective prenatal cohort, congenital malformations were not related to increased concentrations of 10,11-epoxide, or high 10,11-epoxide to carbamazepine concentration ratios in maternal serum or urine (§ 2.7).

Neural tube closure was delayed in rat embryos, which were cultured in vitro in the presence of serum of epileptic patients with low sodium concentrations, presumably associated with carbamazepine use. Sodium, potassium, calcium, chloride, inorganic phosphorus, uric acid, urea nitrogen, creatinine, (and total protein and albumin only in maternal serum) were determined in maternal serum and amniotic fluid. No correlation was found between any of the measured parameters and the occurrence of congenital malformations, although no neural tube defects associated with carbamazepine use, not confounded with concomitant valproate use, were observed. Only one patient with normal pregnancy outcome showed a mild hyponatraemia in early and mid pregnancy (§ 2.8).

Finally, in chapter 3 consensus guidelines for counselling women with epilepsy are given. Female patients who wish to become pregnant should be informed about the two to three fold increased risk of congenital malformations, but it should be pointed out that the woman has a chance over 90% of having a normal child. Furthermore, the possibilities, limitations as well as the parent's attitude towards prenatal diagnosis should be discussed. Patients who are dependent on antiepileptic drug treatment for the control of epilepsy should continue to receive their treatment, because of the dangers of abrupt withdrawal of antiepileptic drug therapy, *i.e.* recurrence or increase in seizure frequency or even status epilepticus, as well as the possible adverse effects of such increase in seizure frequency on the fetus. Whenever possible, monotherapy with the lowest effective dose should be used. Patients who have been seizure free for several years, and those with only occasional seizures, discontinuation of medication before conception and during the first trimester should be considered. Should there however, be any seizure recurrence or increase in frequency, then antiepileptic drug treatment should be started again. If in fact the patient is already a few weeks pregnant, there are probably no advantages in changing the antiepileptic medication.

SAMENVATTING

Er zijn duidelijke aanwijzingen dat kinderen van moeders met epilepsie, die tijdens het eerste trimester van de zwangerschap blootgestaan hebben aan antiepileptica, een 2 tot 3 maal hoger risico hebben op aangeboren afwijkingen dan kinderen uit de algemene populatie. Dit risico blijkt ook hoger te zijn dan dat voor kinderen van niet behandelde aanstaande moeders en vaders met epilepsie. Dit suggereert dat antiepileptica een rol spelen in de inductie van de waargenomen aangeboren afwijkingen, hoewel dit een punt van discussie blijft daar het type epilepsie van de moeder en de daarvoor benodigde medicatie geen onafhankelijke factoren zijn en zelden een specifieke afwijking gevonden wordt na het gebruik van één bepaald geneesmiddel. De literatuur met betrekking tot deze punten worden besproken in hoofdstuk 1. Tevens wordt ingegaan op een aantal gepostuleerde mechanismen via welke antiepileptica aangeboren afwijkingen zouden kunnen veroorzaken.

Neurale buisdefecten lijken specifiek geassocieerd te zijn met het gebruik van valproaat (geschat risico 1-2%), en carbamazepine (geschat risico 0,9%). Aangezien dit risico 10 tot 20 keer hoger is dan het risico op neurale buisdefecten in de algemene populatie ($\approx$0,05-0,1%) en tevens gelijk is aan het risico voor ouders met een voorgaand kind met een neuraalbuisdefect werd het gebruik van deze middelen tijdens het eerste trimester van de zwangerschap een indicatie voor vruchtwateronderzoek. Een kort overzicht van de regulatie van vruchtwater en de distributie van geneesmiddelen hierin wordt gegeven in paragraaf 1.3.

In samenwerking met de Polikliniek en het Laboratorium voor Prenatale Diagnostiek van het Academisch Ziekenhuis Rotterdam - Dijkzigt werd van november 1985 tot juli 1990 een prospectief cohort onderzoek verricht naar het causale verband tussen antiepileptica-gebruik in de zwangerschap en het optreden van neurale buisdefecten (§ 2.1).

Geëvalueerd werden 297 zwangerschappen van 261 verschillende vrouwen verwezen voor prenatale diagnostiek in verband met chronische antiepileptica medicatie. In 5 van de 92 zwangerschappen (5,4%) met maternaal valproaatgebruik werd spina bifida prenataal gediagnostiseerd; gevallen van anencephalie werden niet waargenomen. Het optreden van spina bifida was geassocieerd met een significant hogere gemiddelde valproaat-dagdosering (500 mg per dag meer). Er was geen verband met het type maternale epilepsie, de medische anamnese, noch de familieanamnese voor epilepsie of neurale buisdefecten. Wanneer valproaatgebruik niet vermeden kan worden lijkt het teratogene risico verminderd te kunnen worden door reductie van de dagdosering en de dosis per keer (§ 2.2).

Onder de in Nederland bekende neurale buisdefecten die geassocieerd zijn met maternaal antiepileptica-gebruik (34), blijkt spina bifida ook veel vaker voor te komen dan anencephalie (33:1). Dit veronderstelt een specifieke associatie met caudale sluitingsdefecten.

De meeste neurale buisdefecten na valproaatgebruik zijn ernstige open defecten, welke vaak geassocieerd zijn met hydrocephalus. Ook andere middenlijndefecten, zoals hypospadie (2), hypertelorism (2), een ventrikel-septum defect en diverse intracerebrale aanlegstoornissen werden vaker gezien (§ 2.3).

Alle vijf spinale defecten waargenomen in onze prospectieve studie waren prenataal gediagnostiseerd met α-fetoproteine bepaling in vruchtwater en bevestigd met acetylcholinesterase electroforese. Echter in slechts twee van de vijf gevallen was het maternaal serum α-foetoproteine verhoogd. Geïsoleerd bepalen van maternaal serum α-fetoproteine als diagnosticum voor neurale buisdefecten, in vrouwen die valproaat gebruiken, is onbetrouwbaar (§ 2.4).

Aangezien continuering van de medicatie tijdens de zwangerschap vaak onontkoombaar is, dient gezocht te worden naar de medicatie met het laagste teratogene risico. Het vaststellen van andere mogelijk risico-verhogende factoren zou bij erfelijkheidsadvies kunnen helpen. In dit cohort waren 76% van de vrouwen ingesteld op monotherapie. Het meest gebruikte geneesmiddel was carbamazepine (54%), zowel alleen (38%) als in combinatie met andere antiepileptica (16%). Van alle 303 borelingen had 6.9% een aangeboren afwijking, waaronder spina bifida (6), aangeboren hartafwijkingen (2), hypospadie (2) en een hazelip of gespleten gehemelte (3). Na uitsluiten van de met valproaat-geassocieerde spina bifidas bleken een partiële epilepsie bij de moeder en een positieve maternale zowel als paternale familieanamnese voor epilepsie relatief vaker voor te komen in de families van een kind met aangeboren afwijkingen. In die families met een kind met een aangeboren afwijking bleken ook iets vaker voorgaande kinderen een aangeboren afwijking te hebben. Of dit effect een genetische predispositie voor bepaalde afwijkingen reflecteert of een predispositie voor teratogene effecten, indien gedurende opeenvolgende zwangerschappen dezelfde medicatie gebruikt is, blijft onderwerp voor toekomstig onderzoek. Geen van de antiepileptica toonde een duidelijk hoger risico ten opzichte van alle andere gebruikte antiepileptica (§ 2.5).

Om de hypothese te testen of verschillen in metabolisme van zowel valproaat als carbamazepine een verklaring vormen voor de waargenomen verschillen tussen individuen in optreden van aangeboren afwijkingen, werden de concentraties van voornoemde middelen en een aantal van hun metabolieten bepaald in vruchtwater, en maternaal serum en 24 uurs urine. In 52 zwangerschappen van vrouwen met chronisch valproaatgebruik, hebben wij de concentraties van valproaat en 13 van haar metabolieten bepaald met gaschromatografie-massa spectrometrie. Concentraties in vruchtwater van valproaat en verschillende van haar metabolieten ((E)Δ^2-valproaat, (2E,3'E)$\Delta^{2,3'}$-valproaat en 3-keto-valproaat) correleerden met totale concentraties van valproaat alsook met de niet-eiwit gebonden concentraties van valproaat in maternaal serum. Wij veronderstellen dat het vruchtwater zich gedraagt als een diep compartiment. De resultaten geven verder aan dat tijdens het eerste en vroege tweede trimester van de zwangerschap de ß-oxydatie van valproaat vermindert. In zwangerschappen met foetale neurale buisdefecten waren significant hogere valproaat-dagdoseringen gebruikt die resulteerden in hogere valproaat-concentraties in maternaal serum. In de metabolietpatronen in maternaal serum, 24 uurs urine en vruchtwater konden geen significante verschillen worden aangetoond. Deze resultaten ondersteunen de hypothese dat

valproaat zelf en niet een van de gemeten metabolieten het teratogene agens is (§ 2.6).

Voor wat betreft het gebruik van carbamazepine, vooral in polytherapie, zijn epoxide-metabolieten in verband gebracht met het optreden van aangeboren afwijkingen. De afbraak van carbamazepine via het 10,11-epoxide - 10,11-diol is bepaald met behulp van hoge-druk-vloeistof-chromatografie, in 100 zwangerschappen (inclusief 7 met aangeboren afwijkingen) waarbij carbamazepine gebruikt was, alleen of in combinatie met andere antiepileptica. In maternaal serum nam de 10,11-epoxide concentratie toe met toenemende dosis. Comedicatie met fenobarbital resulteerde in lagere 10-11-epoxide concentraties in maternaal serum en een hoger percentage van de dosis die als 10,11-diol werd teruggevonden in de urine. Polytherapie met valproaat resulteerde in hogere 10-11-epoxide concentraties in maternaal serum in combinatie met iets lagere carbamazepine concentraties. Met valproaat of clonazepam comedicatie werd een lager percentage van de dosis in de urine teruggevonden als 10,11-diol. In vruchtwater, waren de concentraties van carbamazepine en haar hoofd-metabolieten in de meeste patiënten 2 tot 2,5 maal hoger dan de niet-eiwit gebonden concentraties in maternaal serum. Bij carbamazepinegebruik in de zwangerschap konden de aangeboren afwijkingen in deze studie niet gerelateerd worden aan de 10,11-epoxide concentraties of de 10,11-epoxide/carbamazepine ratio in maternaal serum of urine (§ 2.7).

Uit in-vitro experimenten met ratte-embryo's was gebleken dat de sluiting van de neurale buis vertraagd was bij kweek in aanwezigheid van serum van epilepsiepatiënten met lage natrium-concentraties in associatie met carbamazepinegebruik. Wij hebben daarom ook natrium, kalium, calcium, chloride, anorganisch fosfaat, urinezuur, ureum stikstof, creatinine, (en totaal eiwit en albumine uitsluitend in maternaal serum) bepaald in maternaal serum en vruchtwater. Er werd geen verband gevonden tussen een van de gemeten parameters en het voorkomen van aangeboren afwijkingen, hoewel er zich in deze studie geen neurale buisdefecten hadden voorgedaan na carbamazepinegebruik zonder gelijktijdig valproaatgebruik. Slechts één patiënt met een normale zwangerschapsuitkomst vertoonde een milde hyponatriëmie zowel in het eerste als tweede trimester van de zwangerschap (§ 2.8).

Tenslotte worden in hoofdstuk 3 aanbevelingen gegeven voor voorlichting ten aanzien van epilepsie en zwangerschap. Vrouwelijke epilepsiepatiënten met zwangerschapswens dienen geïnformeerd te worden over het twee- tot drievoudig verhoogde risico op aangeboren afwijkingen. Het dient benadrukt te worden dat de vrouw een kans van meer dan 90% heeft op het krijgen van een gezond kind. Verder dienen de mogelijkheden en beperkingen alsmede de houding van de ouders ten opzichte van prenatale diagnostiek en selectieve abortus besproken te worden. Patiënten die afhankelijk zijn van antiepileptica ter voorkoming van insulten dienen hun medicatie te continueren, aangezien abrupt stoppen met antiepileptica het risico van terugkeren of toenemen van aanvallen tot zelfs status epilepticus met zich mee kan brengen en dit mogelijk nadelige effecten voor de foetus kan opleveren. Monotherapie met de laagst mogelijke dosis verdient de voorkeur. Patiënten die reeds gedurende een aantal jaren aanvalsvrij zijn en zij die slecht zelden een insult door maken zouden kunnen proberen hun medicatie te stoppen, voor de conceptie en tenminste gedurende het eerste trimester. Bij een terugkeren van de aanvallen of een toename in aanvalsfrequentie dient de medicatie echter weer hervat te worden. Indien een patiënt reeds enkele weken zwanger is, lijken er geen voordelen te zijn om de gebruikte antiepileptische medicatie alsnog te wijzigen.

CURRICULUM VITAE

January 11,	**1962**	Juliette Geertruida Caecilia Omtzigt, born in Haarlem, The Netherlands (daughter of Martin Omtzigt and José Versteeg)
1974-1980		Education at the "Alkwin College" in Uithoorn.
1980-1986		Student at the Subfaculty of Pharmacy, University of Amsterdam, The Netherlands
January 5,	**1983**	Bachelor degree in Pharmacy, University of Amsterdam
July	**1984 -**	Laboratory of Toxicology, University Hospital Leiden, The Netherlands
February	**1985**	(Prof. Dr. F.A. de Wolff), "On-line RP-HPLC determination of amitriptyline and metabolites in perfusate and cardiotoxicity of (E)-10-OH-nortriptyline in the isolate heart according to Langendorff."(under the supervision of Dr. P.M. Edelbroek)
September	**1984 -**	Teaching assistant (5/10), Department of Pharmaceutical Chemistry (Prof. W. Soudijn),
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February	**1987 -**	Research assistant (A.I.O), Department of Cell Biology and Genetics,
May	**1991**	Faculty of Medicine and Health Sciences, Erasmus University Rotterdam (Prof. Dr. H. Galjaard) The study was caried out under the supervision of Prof. Dr. D. Lindhout)
1987		A.I.O - curriculum Clinical Research Investigator, Erasmus University Rotterdam
April	**1989 -**	Institute of Toxicology and Embryopharmacology, Free University Berlin, Germany
December	**1989**	(Prof. H. Nau, PhD) "Changes in folate metabolism in relation to the induction of neural tube defects by valproate in mice".
1991-1992		Post-graduate education in Toxicology, a cooperated initiative of the Universities of Wageningen - Utrecht - Leiden - Amsterdam, The Netherlands
May	**1991 -**	Research associate, Foundation of Clinical Genetics Rotterdam, The Netherlands
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STELLINGEN

behorend bij het proefschrift:

"Epilepsie, antiepileptica en aangeboren afwijkingen",

door

J.G.C. Omtzigt

Erasmus Universiteit Rotterdam,

21 oktober 1992

I. Valproaatgebruik tijdens het eerste trimester van de zwangerschap is specifiek geassocieerd met spina bifida en niet met anencephalie.

II. Het risico op spina bifida na valproaatgebruik neemt toe met toenemende dosis.

III. De functie van neurotransmitters als regulatoren van morfogenetische processen verdient meer aandacht.

IV. Naast de vetoplosbaarheid bepalen ook de functionele groepen van een stof de mate van accumulatie in vetweefsel.
(Bickel M.H.; Progr Drug Res 1984;28:273-303)

V. Boven de 65 jaar hebben vrouwen met hoge cholesterolspiegels de beste overlevingskans.
(Law MR, Thompson SG. Cancer Causes Control 1991;2:263-61, MRC Working party. Br Med J 1992;304:405-12)

VI. Het door de zorgverzekeraars gemaakte onderscheid in de verstrekking van chemische en mechanische anticonceptiemiddelen is obsoleet.

VII. Door het plaatsen van een "schutting" lijkt de architect zelf al aan te geven dat het gebouw erachter beter aan het oog onttrokken kan worden.
("De Stopera", Royal Damcenter; architect Cees van Dam)

VIII. Kleine criminaliteit bestaat niet.

IX. Moraal is uiteindelijk vooral een overlevingsstrategie.
(Dupuis HH, Opzij 1992;20:46-51)

X. Intermenselijke conflicten zijn heden ten dage een grotere reden tot ziekteverzuim dan arbeidshygiënische of ergonomische problemen.

XI. De voorstanders van de mini-rotonde moeten nog nooit een vrachtwagen met aanhanger gezien hebben.