readily be excluded but it may be relevant in this respect that increasing the medium [Ca^{2+}], which reverses the depressant effect of Mg^{2+} on the synaptic release of transmitters from cholinergic[26] and probably also from adrenergic[27] terminals, did not reverse the effects of Mg^{2+} on the amino acid-induced responses of ventral roots. Indeed, increases in [Ca^{2+}] also produced depressant effects on these responses which were additive with, but considerably weaker than those produced by Mg^{2+}.

The third possibility would imply the involvement of different types of post-synaptic receptors for excitatory amino acids. In this case, kainate and quisqualate might be considered to act almost exclusively on Mg^{2+}-insensitive receptors, and N-methyl-D-aspartate on Mg^{2+}-sensitive receptors. Other amino acids may be presumed to act to a greater or lesser extent on both types of receptors. Such an hypothesis would accord with the different actions of DL-homocysteate and L-glutamate on cat spinal motoneurones[13], and with the differential sensitivity of different groups of cat spinal neurones to L-glutamate and L-aspartate[11] and especially to kainate and N-methyl-D-aspartate[12].

It may be significant that the DR-VRP is markedly attenuated by such low concentrations of Mg^{2+} in the presence of normal [Ca^{2+}]. We have shown that this depression is only partially reversed by increasing the [Ca^{2+}] of the medium, as also noted by Katz and Miledi[28]. Thus, not all of the depression of the DR-VRP by Mg^{2+} may be due to inhibition of transmitter release[26]. This raises the possibility that Mg^{2+}-sensitive transmitter receptor sites may be involved in the generation of the DR-VRP.

26 J. Del Castillo and L. Engbaek, J. Physiol., Lond. *124*, 370 (1954).
27 H. Kuriyama, J. Physiol., Lond. *175*, 211 (1964).
28 B. Katz and R. Miledi, J. Physiol., Lond. *168*, 389 (1963).

Effect of lanthanons on substrate-induced difference spectra in rat liver microsomes

P. Arvela[1], O. Grajewski, B. v. Lehmann and E. Oberdisse

Department of Pharmacology, Free University of Berlin, Thielallee 69/73, D–1000 Berlin 33 (Berlin West), 21 September 1976

Summary. Intravenously administered light lanthanons change spectral interactions in rat liver not only by decreasing the concentration of cytochrome P-450, but they also cause a qualitative change in the cytochrome P–450 molecule or its microenvironment.

The lanthanons are divided into light and heavy elements, having different biological effects. Intravenously administered light lanthanons (Lanthanum–Samarium) cause fatty infiltration of the liver[2] and impairment of the drug metabolism[3]. The heavy lanthanons (Europium–Lutetium) cause only focal necroses of the liver without changes in the lipid concentration[4] or the metabolic activity[5]. Light lanthanons have been shown to decrease the amount of cytochrome P-450 (Cyt. P-450) to about 50% of its initial value, whereas the decrease caused by the heavy lanthanon, Erbium, is only 10%[5]. The addition of various substances, such as hexobarbital or aniline, to liver microsomes induces difference spectra which are considered to be indicative of the binding to the Cyt. P-450, the terminal oxidase of the microsomal electron transport chain[6,7].

The aim of the first part of this study was to compare the effects of a light lanthanon, Cerium (Ce), and a heavy one, Erbium (Er), on the substrate binding capacity of the Cyt. P-450 in the microsomal fraction of rat liver. Possible changes in spectral interactions may indicate qualitative differences in the function of the decreased Cyt. P-450 caused by lanthanons. The second part of our study was based on the report of Lehmann et al.[8] who found differ-

1 P. Arvela is a fellow of the Alexander-von-Humboldt-Stiftung.
2 F. Snyder and N. Stephens, Proc. Soc. exp. Biol. Med. *106*, 202 (1961).
3 P. Arvela and N. T. Kärki, Experientia *27*, 1189 (1971).
4 G. Magnusson, Acta Pharmac. Toxic. *20*, suppl. 3 (1963).
5 P. Arvela and N. T. Kärki, Symposium on Drug-Induced Metabolic Changes (I. Congress of the Hungarian Pharmac. Soc., Budapest 1971).
6 G. I. Mannering, in: Fundamentals of Drug Metabolism and Drug Disposition. The Williams and Wilkins Co., Baltimore 1971.
7 A. H. Conney, Pharmac. Rev. *19*, 317 (1967).
8 B. v. Lehmann, E. Oberdisse, O. Grajewski and H.-R. Arntz, Archs. Toxic. *34*, 89 (1975).

Table 1. The liver weights and the amounts of Cyt. P-450 and b_5 in variously treated groups of male rats ($\pm$ SD)

	Liver weight (g)	Rel. liver weight (g)	Cyt. P-450 nmoles/mg prot.	Cyt. b_5 nmoles/mg prot.
Group I Controls	9.03 $\pm$ 1.18	4.47 $\pm$ 0.12	0.556 $\pm$ 0.150	0.346 $\pm$ 0.141
Group II Ce	12.49 $\pm$ 0.80*	5.36 $\pm$ 0.29*	0.348 $\pm$ 0.042*	0.182 $\pm$ 0.044*
Group III Er	9.19 $\pm$ 1.11	4.19 $\pm$ 0.37	0.520 $\pm$ 0.138	0.386 $\pm$ 0.061

*$p < 0.01$

ences in the phospholipid distribution within liver subcellular fractions after treatment with another light lanthanon, Praseodymium (Pr). Because it is known that the Cyt. P-450 needs a phospholipid environment for proper function[9], we tested the effect of Pr on the binding capacity of Cyt. P-450 in the smooth (SER) and rough (RER) endoplasmic reticulum.

Materials and methods. In the first part of the study, male Sprague-Dawley rats weighing 170–220 g were used. They were divided into 3 groups of 6 each: group I, controls; group II, receiving i.v. Ce (2 mg/kg) as chloride in saline, and group III, receiving Er (2 mg/kg) as in the group II. The animals were killed 3 days after injection, when according to our earlier studies the toxic effects were most pronounced[3]. Livers were removed, rinsed with ice-cold 0.1 M Tris-HCl buffer, pH 7.4, and homogenized in 4 volumes of the same buffer with a Potter-Elvehjem type homogenizer. The homogenate was centrifuged at 12,000 $\times$g and 100,000 $\times$g for 20 and 60 min respectively. The microsomes were resuspended in the same buffer corresponding to 1 g liver/ml. In the second part of study, adult female Wistar rats weighing 150–180 g were pretreated as described before[8]. They received 3 mg/kg Pr i.v. as nitrate in saline and were killed 2 days after injection. To separate SER and RER the supernatant obtained after 12,000 $\times$g was submitted to a discontinuous gradient centrifugation according to Fleischer and Kervina[10]. The purity of the fractions was checked by electron microscopy.

For determination of the special interactions, the fractions were suspended in the same buffer to yield a protein concentration of about 2 mg/ml. To obtain sufficient material for measuring the spectral interactions in SER and RER, livers of the 2 animals were pooled. Spectra were measured at room temperature in 1 cm cells with an Aminco-Bowman spectrophotometer. The extent of the type I and type II spectral changes was measured by the difference in the absorption between the wavelenghts of minimum and maximum absorption. With hexobarbital, there is a decrease in optical density around 420 nm and an increase around 390 nm (type I spectral change), with aniline there is an increase around 430 nm and a decrease around 390 nm (type II spectral change). The cytochromes b_5 and Cyt. P-450 were measured according to the method of Omura and Sato[11]. Protein was assayed by the Lowry reaction[12]. Student's t-test was used to calculate the significance of the results.

Results and discussion. The liver weights and the amounts of the Cyt. P-450 and b_5 of the first part of our study are given in table 1. The liver weights of Ce-treated rats were significantly higher than those of controls or Er-treated rats. This increase is mostly due to the accumulation of fat in the livers of rats receiving light lanthanons[13]. When examined microscopically, the livers of Ce-treated rats showed clear signs of fatty degeneration. This is contradictory to the statement of Snyder et al.[14] that the light lanthanons cause fatty liver only in females. The discrepancy may be due to the different rat strain used. In the livers of Er-treated rats, neither fatty infiltration nor severe necroses could be seen. The liver protein concentration was decreased to about 84% of corresponding controls only in the Ce-treated rats. This is in accordance with findings of Schurig et al.[15] who reported a 30% decrease of liver proteins in female rats treated with another light lanthanon, Pr, and fasted for 3 days. The amounts of both cytochromes were significantly decreased in the Ce-pretreated group.

The spectral interactions with hexobarbital and aniline ligands in the microsomal fraction are shown in table 2. Because the decrease in the Cyt. P-450 content after Ce- or Pr-treatment is significant, the A_{max}-values are corrected with the corresponding amounts of Cyt. P-450. After this correction, Ce still significantly inhibits the binding of both ligands to Cyt. P-450. Although the binding capacity of Cyt. P-450 from Er-treated rats tends to be lower than in controls, the changes were not significant. The K_s-values are increased for type I spectra in both lanthanon-treated groups, but for type II spectra induced by aniline the increase is only seen in the Ce-treated animals. It has been proposed that the type I binding occurs at the protein moiety and type II at the heme iron of Cyt. P-450[6]. In our study, no great differences between these 2 binding sites could be seen, but it seems that lanthanons act primarily on the protein structure of the Cyt. P-450 molecule.

Recently Lehmann et al.[8] reported differences in the subcellular distribution of phospholipids induced by Pr in female rats. Because the phospholipids are found to be closely associated with the type I binding site, we measured the binding capacity of hexobarbital and aniline ligands in the 2 liver subcellular fractions, SER and RER. The spectral interactions in these subfractions are shown in table 3. According to Lehmann et al.[8], the Pr-induced decrease in phospholipid and Cyt. P-450 contents occurs

Table 2. Effect of Ce and Er on the with hexobarbital (type I) and aniline (type II) induced difference spectra

	Hexobarbital A_{max}	K_s	Aniline A_{max}	K_s
Group I Controls	18.0 $\pm$ 1.9	87	20.4 $\pm$ 1.2	910
Group II Ce	13.4 $\pm$ 1.8*	175	16.8 $\pm$ 2.6*	1820
Group III Er	13.3 $\pm$ 5.3	118	17.8 $\pm$ 6.6	910

The values are given in A_{max}/nmoles Cyt. P-450 $\times 10^{-3}$ $\pm$ SD. K_s in μM. *$p < 0.01$.

Table 3. The spectral interactions in SER and RER subfractions of rat liver after pretreatment with Pr

		Hexobarbital	Aniline
SER	Controls (4)	5.6 $\pm$ 0.2	15.8 $\pm$ 0.1
	Pr (4)	3.1 $\pm$ 0.2	12.8 $\pm$ 0.1
RER	Controls (4)	4.8 $\pm$ 0.5	11.6 $\pm$ 0.5
	Pr (3)	6.4 $\pm$ 0.2	23.5 $\pm$ 3.8

The values are expressed as A_{max}/nmoles Cyt. P-450 $\times 10^{-3}$ $\pm$ SD.

9 H. W. Strobel, A. Y. H. Lu, J. Heidema and M. J. Coon, J. biol. Chem. *245*, 4851 (1970).
10 S. Fleischer and M. Kervina, Meth. Enzym. *31*, part A (1974).
11 T. Omura and R. Sato, J. biol. Chem. *239*, 2370 (1964).
12 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, J. biol. Chem. *193*, 265 (1951).
13 F. Snyder, E. A. Cress and G. C. Kyker, J. Lipid. Res. *1*, 125 (1959).
14 F. Snyder, E. A. Cress and G. C. Kyker, Nature *185*, 480 (1960).
15 R. Schurig and E. Oberdisse, unpublished results.

only in the RER fraction. The disturbances in the spectral interactions are also most prominent in this fraction. When, however, the drastic fall in Cyt. P-450 content of RER (72%) is taken into account, a significant qualitative effect of Pr can only be seen in the SER. Liver microsomes are known to contain multiple forms of Cyt. P-450. It is possible that the apparent increase in the binding capacity occurring in RER is due to an enrichment of a more active form in the remaining part (28%) of this cytochrome.

Our results indicate that light lanthanons do not cause changes in the spectral interactions only by decreasing the concentration of Cyt. P-450, but also cause a qualitative change in the Cyt. P-450 molecule or in this microenvironment. There are differences in the binding capacity between SER and RER subfractions. This may indicate that more than one kind of Cyt. P-450 molecule is reacting, or that some endogenous substrates may partly mask one or more binding sites of the cytochrome. The reaction of lanthanons with the lipid factor may, on the other hand, disturb the access of ligands to the cytochrome molecule[6,16].

16 The authors wish to thank Mrs Iris Baar, Mrs Brigitte Schröder and Mrs Brunhilde Enkemann for their valuable technical assistance.

Chlorpromazine: A potential physiological teratogen

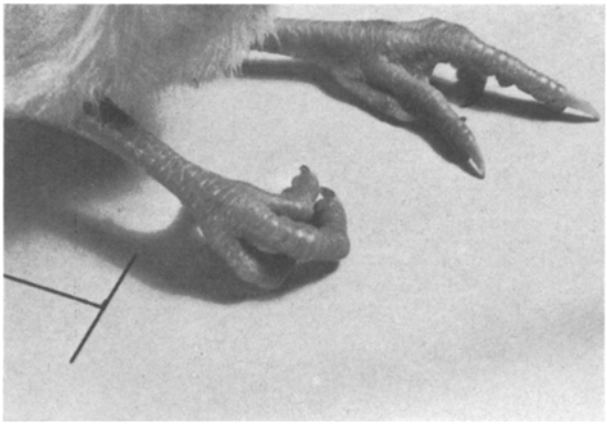
C. J. Sherry

Department of Biology, College of Science, Texas A & M University, College Station (Texas 77843, USA),
14 September 1976

Summary. Chlorpromazine, a drug commonly administered as an antiemetic during pregnancy, when administered prenatally to chick embryos, was associated postnatally with a 'curled toe' anomaly (ED 50% of 88 µg/egg for the 4-day-embryo).

Chlorpromazine (CPZ) can and does cross the placental barrier in both animals and man[1-7]. It is routinely administered to human females during the first trimester of pregnancy to control nausea and vomiting (i.e. 25 mg/kg)[8]. It has been reported that administration of large doses (i.e. 50–150 mg/day to 8000 mg/10 days) to control maternal depression and/or psychotic behavior has no neonatal effects[9-12]. However, a number of extrapyramidal dysfunctions have been reported following prenatal human exposure to relatively large doses of CPZ[13-15]. In addition, in rodents, if CPZ is administered prior to gestation, no births take place[16]. If CPZ treatment is suspended prior to gestation, a few litters are born, but each litter has fewer pups than expected[16-19] and the pups that are born are small for dates[16]. This data suggest that it would be important to determine if CPZ is a physiological and/or anatomical teratogen. Since the developing chicken embryo responds to all known teratogens[20,21], it was decided to determine the effect of prenatal exposure to CPZ on the postnatal development of the chicken.

Methods. 120 eggs were obtained from the Colonial Poultry Farms and refrigerated prior to incubation to insure that they would be at the same developmental age[22]. Following the technique of Karnofsky[21], 6 groups



The 'curled toe' anomaly. Note that all of the phalanges on 1 limb and 2 sets of phalanges on the other are involved. The neck and upper limb muscle are not involved in the anomaly. In addition, cloacal evacuation is normal.

1 S. E. F. O Donoghue, Nature *229*, 124 (1971).
2 O. Pilkonen, M. Vorne, P. Jouppila and N. T. Karki, Acta pharmac. tox. *29*, 284 (1971).
3 O. Pelkonen, M. Vorne and N. T. Karki, Acta physiol. scand., suppl. 330, 69 (1969).
4 G. J. Alexander, S. Machiz and R. B. Alexander, in: Drugs and Fetal Development, p. 151. Ed. M. A. Klingberg, A. Abramovici and J. Chemke. Plenum, New York 1972.
5 V. Francova, Z. Franc and I. Lamplova, Physiol. bohemoslov. *12*, 150 (1963).
6 V. Francova, Z. Franc, Z. Votava and I. Lamplova, Čslká Gynek. *28*, 301 (1963).
7 S. E. Sjöstrand, G. B. Cassano and E. Hansson, Arch. int. Pharmacodyn. *7*, 34 (1965).
8 M. E. Jarvik, in: The Pharmacological Basis of Therapeutics. Ed. L. S. Goodman and A. Gilman. Macmillan, New York 1970.
9 E. B. Kris, in: Recent Advances in Biological Psychiatry, p. 4. Plenum, New York 1962.
10 E. B. Kris, Curr. Ther. Res. *7*, 785 (1965).
11 E. B. Kris and D. M. Carmichael, Psychiat. Q. *685* (1957).
12 J. E. Hammond and P. A. Toseland, Archs Dis. Childh. *45*, 139 (1970).
13 W. Levy and K. Wisniewski, N. Y. State J. Med. *74*, 684 (1974).
14 R. M. Hill, M. M. Desmond and J. L. Kay, J. Pediat. *69*, 589 (1966).
15 A. Tamer, J. Pediat. *74*, 479 (1969).
16 G. J. Alexander, S. Machiz and R. B. Alexander, in: Drugs and Fetal Development, p. 151. Plenum, New York 1972.
17 J. M. Ordy, A. Latanick, R. Johnson and L. C. Massopust, Proc. Soc. exp. Biol. Med. *113*, 833 (1963).
18 L. Meyler (Ed.), in: Side Effects of Drugs, p. 60. Excepta Medica Foundation, Amsterdam 1966.
19 J. R. Beall, Toxic. appl. Pharmac. *21*, 230 (1972).
20 D. O. E. Gebhardt, Adv. Terat. *5*, 97 (1972).
21 D. A. Karnofsky in: Teratology: Principles and Techniques, p. 194. Univ. Chicago Press, Chicago 1965.
22 Armed Forces Institute of Pathology, Manual of Histologic and Special Staining Technics. Government Printing Office 1957.