tioned medium (Gibco, Grand Island, New York, USA). After 8 days of incubation at 37 °C in a humidified atmosphere at 5% CO, 3 dishes per point were scored and all aggregates of more than 40 cells were counted as colonies.

Results and discussion. The results are shown in the figure. At concentrations of up to 250 µg/ml neither drug altered CFU-GM proliferation. Significant growth inhibition appeared at 500 µg/ml, and virtually no colonies were counted at 1000 µg/ml. 50% inhibition of growth was present at a concentration of about 600 µg/ml of cimetidine and of about 800 µg/ml of ranitidine.

The mechanism of this inhibition pattern is not clear. The blocking of H_2 receptors on stem cells seems very unlikely since ranitidine would have inhibited colony formation at relatively low concentrations if this were the case. In fact both drugs displayed the same inhibition curve. It must therefore be supposed that other mechanisms are responsible for the toxicity of both compounds for CFU-GM. On the other hand it is by no means clear whether our data mean that the risk of myelo-suppression is reduced by using ranitidine, which is clinically effective at lower blood levels and presumably bone marrow microenvironment levels than cimetidine. In fact 50% inhibition of gastric secretion in man is obtained at a plasma concentration of 0.1 µg/ml of ranitidine and at a concentration of 0.5 µg/ml of cimetidine[13-15]. Quite apart from the obvious difficulty of translating in vitro into in vivo data, inhibition was, in effect, only observed at drug concentrations higher than those seen therapeutically. Therefore, although, as far as we know, no cases of myelosuppression have as yet been reported with ranitidine, it would seem to be too early to declare it non-toxic.

1 Acknowledgments. This work was supported by CNR, Rome, PFCCN and by AIRC, Milan.
2 Isemberg, J.I., Ann. intern. Med. *84* (1976) 212.
3 Posnett, D.N., Stein, R.S., Graber, S.E., and Krantz, S.B., Archs intern. Med. *139* (1979) 584.
4 Freston, I.W., Ann. intern. Med. *90* (1979) 264.
5 Byron, J.W., Expl. Hemat. *8* (1980) 256.
6 Volkin, R.L., Shadduck, R.K., Winkelstein, A., Ziegler, Z.R., and Selker, R.G., Blood *54* (1979) 214a.
7 Price, G.B., and Krogsrurd, R.L., in: Differentiation of normal and neoplastic hematopoietic cells, p.371. Eds B. Clarkson, P. Marks and J.E. Till. Cold Spring Harbor Conference on Cell Proliferation, 1978.
8 Fitchen, J.K., and Koeffler, H.P., Br. J. Haemat. *46* (1980) 361.
9 Peden, N.R., Richard, D.A., Saunders, J.H.B., and Wormsley, K.B., Lancet *2* (1979) 199.
10 Britain, P.T., and Daly, M.J., Scand. J. Gastroent. *16* suppl.69 (1981) 1.
11 Louis, W.J., Nihaly, G.W., Hanson, R.G., Anderson, A., Mc Neil, J.J., Yeomans, N.D., and Smallwood, R.A., Scand. J. Gastroent. *16*, suppl.69 (1981) 11.
12 Aglietta, M., Piacibello, W., and Gavosto, F., Cancer Res. *40* (1980) 2507.
13 Burland, W.L., Duncan, W.A.M., Hesselb, O.T., Mills, J.G., Sharpe, P.C., Haggie, S.J., and Willie, J.H., Br. J. clin. Pharmac. *2* (1975) 481.
13 Brimblecombe, R.W., and Duncan, W.A., in: Cimetidine, proceedings of the second international symposium on histamine H_2 receptor antagonist, p.56. Eds W.L. Burland and M.A. Simkins. Excerpta Medica, Amsterdam 1977.
14 Woodings, E.P., Dixon, G.T., Harrison, C., Carey, P., and Richards, I., Gut *21* (1980) 187.

0014-4754/85/030375-02$1.50 + 0.20/0

Influence of phenobarbital and TCDD on the hepatic metabolism of TCDD in the dog

H. Poiger and Ch. Schlatter

Institute of Toxicology, Swiss Federal Institute of Technology and University of Zürich, CH-8603 Schwerzenbach (Switzerland), 21 February 1984

Summary. The influence of phenobarbital and TCDD pretreatment on the formation and biliary excretion of TCDD-metabolites following single doses of ^{3}H-TCDD was investigated. Without pretreatment, 24.5% of the absorbed ^{3}H-TCDD dose was excreted in the bile within 110 h. Phenobarbital did not influence this rate, whereas a single dose of 10 µg of unlabeled TCDD/kg b.wt nine days earlier resulted in a doubling of the amount of radioactive material eliminated in the bile (47.4%).
Key words. TCDD; metabolism; biliary excretion; phenobarbital; stimulation.

The pharmacokinetics of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in rats, guinea pigs and hamsters have been reported[1-4]. Depending on the species, the eliminiation half life of this compound was reported to vary between two and about four weeks. Feces contained most of the material excreted, some of which probably arose from hepato-biliary excretion. Bile of rats and hamsters has been shown to contain metabolites of TCDD[3,5,6]. Recently we reported that biliary excretion of TCDD-metabolites in the dog was considerable and identified the structure of some of the biotransformation products[7]. Preliminary observations suggested that TCDD increases its own hepatic metabolism and excretion, but the experimental design of the earlier study, in which large doses of TCDD were given repeatedly, was not suitable for studying these effects. In addition, data on the intestinal absorption and excretion in urine in the dog were lacking. Therefore this work was carried out under well-defined conditions of pretreatment and using low doses of the dioxin, which allowed a determination of the radioactive material in the excreta without excessive risk in handling and measuring samples.

Materials and methods. Chemicals. ^{3}H-labeled TCDD (sp. act. 40 Ci/mmole; source A. Kende, Rochester, NY) was purified by preparative gas chromatography (2 m × 2 mm i.d. all glass column, DC 560, 230 °C), resulting in a radiochemical purity higher than 98% (checked by GC). For dosage it was dissolved in corn oil, yielding solutions containing 0.75 or 0.82 µg of ^{3}H-TCDD/ml, as calculated from radioactivity measurements. Unlabelled TCDD (Dow Chemical, Midland, MI) was used without further purification. It was dissolved in benzene (0.25 mg/ml). Prior to administration 1 ml of this solution was mixed with 5 ml of corn oil and the benzene evaporated by bubbling a stream of nitrogen through the solution.

Animal experiments. A male Boxer dog underwent cholecystectomy with implantation of a Thomas cannula about 18 months before these experiments (age at the time of the surgery was approximately 1 year). In the meantime the dog

was not used for experiments, but trained and used for sampling of bile occasionally. At the beginning of the experiments its weight had reached 25 kg.

Before administering TCDD, the dog was starved for 24 h and then a polyethylene catheter was inserted into the bile duct and connected to a plastic bag, which was strapped on the belly of the animal. We collected the bile continuously for 110 h, changing the plastic bag after the first 14 h and every 24 h thereafter (every 12 h in experiment 4). During the experiments the animal was kept in a metabolism cage, so that urine and feces could be collected separately.

Dosing. Doses were given orally, in that 1 ml of the oily solution was mixed with freshly minced meat; these preparations were immediately ingested by the animal. Four successive experiments were carried out (designated experiments 1–4), leaving time intervals of three weeks between experiments 1 and 2 as well as 2 and 3, and 9 weeks between experiments 3 and 4:

Experiments 1 and 2: Single doses of 96.2 μCi (30 ng/kg, 0.75 μg in total) of ^{3}H-TCDD.

Experiment 3: Single dose of 96.2 μCi of ^{3}H-TCDD after pretreatment with 15 mg/kg/day of sodium phenobarbital for 10 days.

Experiment 4: Single dose of 105.2 μCi (32.8 ng/kg) of ^{3}H-TCDD after pretreatment with a single dose of 10 μg of unlabeled TCDD/kg 9 days earlier.

Sodium phenobarbital encapsulated in gelatine capsules was given orally. The unlabeled TCDD-dose was administered enterally via the Thomas cannula, because high doses of TCDD (about 50 μg/kg) previously had been found to induce vomiting in the dog (the 10 μg/kg dose caused anorexia for about 4 days and slightly apathetic behavior for one week following administration, but beyond that no other signs of toxicity were observed).

Radiochemical techniques. Feces were homogenized with two parts of water-isopropanol (1:1, v/v), aliquots of about 200 mg dissolved in 1 ml of soluene 350 tissue solubilizer (Packard Instrument Company), and decolorized with H_2O_2; bile aliquots of 0.1 ml were mixed with 0.5 ml soluene and 0.5 ml of isopropanol and decolorized with H_2O_2. The samples were then neutralized with glacial acetic acid and counted after addition of 10 ml of emulsifying cocktail. Urine samples were measured after mixing with emulsifying cocktail. For TLC, bile and urine samples were extracted with equal volumes of ethyl acetate, feces were lyophilized and the residues extracted two times with ethyl acetate under ultrasonication. For chromatography, precoated silica gel 60 sheets (0.25 mm layer thickness, Merck, FRG) were used, with carbon tetrachloride serving as developing solvent. Radioactivity was determined in zones of 1 cm after desorption with 2 ml of methanol directly in the scintillation vials and addition of 10 ml of scintillation liquid.

Results and discussion. All experiments were carried out on the same animal, leaving enough time in between for the radioactivity in the excreta to return close to the background levels. The decision to use only one dog for the study, which was made for ethical reasons, might raise questions on the significance of the data. However, we believe that there is enough evidence for what we want to demonstrate.

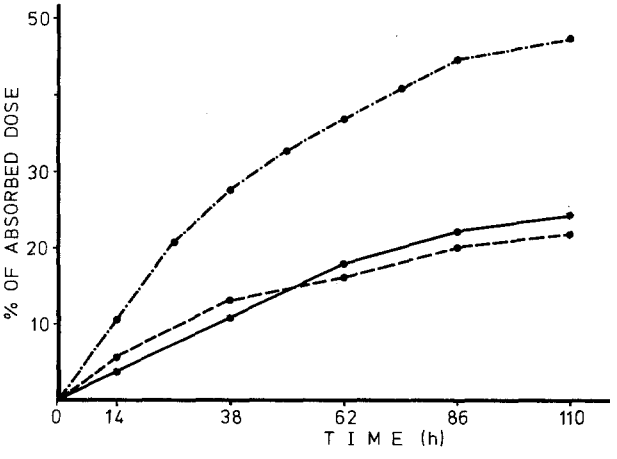
The cumulative biliary excretion of tritium activity, calculated from the absorbed fraction of the dose, is shown in the figure. The profiles of experiments 1–3 were very similar, but a pretreatment with unlabeled TCDD caused a twofold higher elimination of tritium activity with a maximum during the first 38 h after dosage. For ethical and technical reasons sampling of bile was not extended for longer than 110 h. In the table the amounts of ^{3}H-activity eliminated within 110 h in feces, bile and urine are given, calculated from the total dose. Variations in the fecal excretion of radioactivity were most probably a consequence of unequal absorption of TCDD from the intestinal tract (but generally, it should be taken into account that the depletion of the bile could have influenced intestinal absorption of this lipid-soluble compound in all four experiments). Especially in experiment 3 (phenobarbital pretreatment) an increased food intake and defecation was observed which shortened gastrointestinal transit time and thus would explain the lower uptake of TCDD, a substance which adsorbs strongly to all matrices. Feces of the initial 38 h contained quite high amounts of radioactive material, most of it (80–85%) extracting into ethyl acetate and almost entirely cochromatographing with parent TCDD. These amounts probably represent unabsorbed TCDD. Between 38 and 110 h the daily fecal excretion rates were between 0.3 and 3.8% of the dose. Of this radioactivity, 30–35% was extractable with ethyl acetate and only a minor fraction (approximately 10–20% of the extracted radioactivity) cochromatographed with TCDD. Urinary elimination of radioactive material was between 0.1 and 0.5% of the dose/24 h and remained nearly constant during the experimental period. TLC of extracts of bile and of urine did not reveal the presence of any parent TCDD in these excreta.

Whether withdrawal of the bile influenced the elimination half life of the metabolites in the dog is uncertain, because the degree of their enterohepatic circulation is not known. However, we observed an increase in fecal elimination of radioactivity after restoring normal biliary conditions by removing the bile catheter at the end of the experiments. Feces sampled during the 24-h period thereafter contained roughly 50% more tritium activity than the previous samples, which suggests a partial reabsorption of the TCDD-metabolites. Interestingly, it has also been found that the radioactive material extractable from

Cumulative excretions of radioactivity by a dog given single oral doses of 31 or 33.8 ng of ^{3}H-TCDD/kg

Experiment	Pre-treatment	Feces	Bile	Urine
1	Nil	30.2 (25.9)	17.7	2.0
2	Nil	40.6 (37.7)	15.7	0.8
3	Pb	68.9 (64.5)	7.9	0.5
4	TCDD	33.2 (27.8)	34.2	2.0

Numbers give the percentage of the dose excreted within 110 h. Numbers in parentheses give the percentage of the dose excreted within the first 38 h following administration.



Cumulative biliary excretion of TCDD-metabolites by a dog following single oral doses of ^{3}H-TCDD. (All data are referred to the absorbed fraction of the dose.)

———— no pretreatment (mean of two experiments);
— — — — 15 mg of phenobarbital/kg/day, for 10 days;
—·—·—· 10 μg of unlabeled TCDD/kg, nine days earlier.

the bile of dogs is very rapidly cleared from the body of rats[9]. Experiment 2, a repeat of experiment 1, was conducted to check whether possibly induced liver enzymes affect biliary excretion of tritium activity, since in the rat an increase in heptic microsomal P-448-mediated enzyme activities has been reported already at doses of 2 ng of TCDD/kg[8]. Obviously, the small dose of ^{3}H-TCDD was not sufficient for a stimulation of TCDD-metabolism in the dog. From our results it is reasonable to assume that P-448-dependent liver enzymes are involved in the biotransformation of the dioxin. Because of the very distinct effect of TCDD, the most potent out of the group of P-448 inducers, no other classical inducer was investigated. It should be considered that a faster elimination might cause the acute toxicity of TCDD to decrease. In view of data from Beatty et al.[10], who found a higher LD_{50} in male weanling rats pretreated with TCDD (but also with phenobarbital) this seems likely, because the time during which the organism is in contact with this substance certainly plays an important role. Furthermore, available data suggest that TCDD is essentially eliminated from the body only in metabolized form. Whether inducibility of TCDD-metabolism is a phenomenon unique in the dog is a question that deserves further study.

1 Piper, W.N., Rose, J.Q., and Gehring, P.J., Envir. Health Perspec. *5* (1973) 241.
2 Rose, J.Q., Ramsey, J.C., Wentzler, T.H., Hummel, R.H., and Gehring, P.J., Toxic. appl. Pharmac. *36* (1976) 209.
3 Olson, J.R., Gasiewicz, T.A., and Neal, R.A., Toxic. appl. Pharmac. *56* (1980) 78.
4 Gasiewicz, T.A., and Neal, R.A., Toxic. appl. Pharmac. *51* (1979) 329.
5 Poiger, H., and Schlatter, C., Nature *281* (1980) 706.
6 Ramsey, J.C., Hefner, J.G., Karbowsky, R.J., Braun, W.H., and Gehring, P.J., Toxic. appl. Pharmac. *65* (1982) 180.
7 Poiger, H., Buser, H.-R., Weber, H., Zweifel, U., and Schlatter, Ch., Experientia *38* (1982) 484.
8 Kitchin, K.T., and Woods, J.S., Toxic. appl. Pharmac. *47* (1979) 537.
9 Weber, H., Poiger, H., and Schlatter, Ch., Xenobiotica *12* (1982) 353.
10 Beatty, P.W., Vaughn, W.K., and Neal, R.A., Toxic. appl. Pharmac. *45* (1978) 513.

0014-4754/85/030376-03$1.50 + 0.20/0

The use of indentometry to study the effect of agents known to increase skin c-AMP content

A. Hartzshtark and S. Dikstein

Unit of Cell Pharmacology, School of Pharmacy, Hebrew University, P.O. Box 12065, Jerusalem (Israel), 17 January 1983

Summary. Local, externally applied pharmacological agents which are assumed to raise the c-AMP level, decrease the low pressure indentation value of the forehead skin of certain human volunteers.
Key words. c-AMP, skin; indentometry; pharmacological manipulation of mechanical parameter.

Recently low pressure indentometry has been used to measure 'dermal hydration'[1,2] in vivo. This method is used for measuring an aspect of the efficiency of cosmetic treatments and also as a routine diagnostic instrument in medicocosmetic consultation[3]. The measuring system, procedure and instrumentation have been described elsewhere[3]. In essence they are based on low pressure procedures. A light metal measuring rod is counterbalanced so that the net pressure of the system is less than 1 g/cm^2. A circular plate at the end of the rod, having a surface area of 0.2 cm^2, serves as the contact area with the skin. The total weight of the system (including the counterbalance) is 6 g. The measuring rod can be loaded with specially constructed weights, thereby increasing the pressure from the starting pressure to any desired value. The routine final pressure used in our laboratory for in vivo measurements on humans is 10 g/cm^2. The rod is connected to a linear variable differential transformer (LVDT), the output of which is graphically recorded. The routine paper velocity used by us is 6 cm/min. The sensitivity of the measurements is $\pm$ 0.001 cm.

For many reasons[2], the routine measurements are performed on the forehead skin. The patient lies on his/her back with eyes closed and head resting on a wooden plate to prevent recording of breathing and heartbeats. The measuring rod is adjusted so that the plate is in contact with the forehead skin, and the electronic system is zeroed (starting pressure = 1 g/cm^2). The recorder is started and the base line is recorded for about 10–15 sec. The standard weight is now suddenly applied, and the resultant indentation recorded for 6 sec. The weight is then removed and the rebound phase ('elastic recovery') of the skin is recorded for a further 6 sec.

The indentation so measured by low pressure indentometry on the forehead skin is 0.04–0.09 cm. Without treatment, the mean change in the indentation of an individual point during the day is less than $\pm$ 0.003 cm.

It was shown that indentation is usually higher in the so-called 'cosmetically dry skin' cases, and always lower in the 'cosmetically not dry skin' cases, and further, that age increases indentation[3]. We also showed that indentation under our standardized conditions is increased by intradermal hyaluronidase and decreased by water, thus indicating that indentometry reflects the state of ground substance[4].

We wish to discuss here pharmacological agents affecting indentometry. Each substance was tested on four volunteers who had 'high indentation values' (0.06–0.08 cm). Triplicate measurements were carried out at four points on each volunteer (forehead skin). Then the substance was applied to the skin (1 ml during 10 min) and the indentation was recorded at different times; at each time a triplicate measurement at the same point. Statistical evaluation by paired t-test was carried out on the individual changes of each patient.

The table shows that agents known to increase c-AMP in skin by activating adenylate cyclase, such as adenosine[5], the β-agonist isoproterenol bitartarate[6] or the β_2-agonist terbutaline sulfate, or the phosphodiesterase inhibitor papaverine[7], all cause a decrease in indentation at approximately those concentrations at which they are known or supposed to cause an increase in the c-AMP content of the skin[5–7]. This decrease in indentation represents a firmer, younger skin. The final proof that c-AMP is involved can be seen in the table, from the strong effect of 0.1% N^6,O^2,-dibutyryl c-AMP (sodium salt).

The action of the above agents is specific, since 0.3% solution of noradrenaline hydrochloride, histamine hydrochloride, serotonine hydrochloride, guanosine, or N^2,O^2,-dibutyryl c-GMP failed to influence indentation significantly. Each active agent