perfusion were: T ($-0.20\pm2\%$); $+\dot{T}$ ($1\pm2\%$) and $-\dot{T}$ ($6\pm3\%$).

The effect of pentoxifylline on myocardial performance is shown in figure 1. In 9 experiments after 15 min of infusion the only statistically significant changes in mechanical parameters were an increase of $-\dot{T}$ by $21\pm6\%$ and accordingly a decrease of the $+\dot{T}/-\dot{T}$ quotient by 0.20 ± 0.05. In experiments on reserpine pretreated rats, essentially the same response to pentoxifylline was found with a significant increase in $-\dot{T}$ ($15\pm3\%$) and decrease in $+\dot{T}/-\dot{T}$ of (0.13 ± 0.06).

Pentoxifylline infusion increased significantly the level of cAMP and the protein kinase activity ratio (figure 2).

No significant increase of the cGMP level was observed at the end of pentoxifylline infusion.

Discussion. In early studies on the effect of epinephrine in the intact heart it was shown that the observed increase in contractility was accompanied by an increase in the rate of relaxation[28]. This effect was confirmed later with isoproterenol in cat papillary muscles[18,29] and in the perfused rat heart[13]. The effect of β agonists on the relaxation phase was suggested to be mediated through an increase in cAMP since an increased calcium uptake by the sarcoplasmic reticulum was found which was related to an increase in the rate of relaxation following the addition of cAMP[30]. Moreover, in experiments from our laboratory in which the ratio of maximal velocities of contraction to relaxation ($+\dot{T}/-\dot{T}$) was used as an index of relaxation, it was shown that the effect of isoproterenol on relaxation was mimicked by dibutyryl cAMP[13].

The present results show a statistically significant increase in the rate of relaxation ($-\dot{T}$) after 15 min of pentoxifylline infusion with a corresponding decrease in the ratio value ($+\dot{T}/\dot{T}$). This effect can not be attributed to endogenous release of catecholamines since the experiments with hearts depleted of catecholamines by reserpine gave identical results.

Along with the increase in relaxation pentoxifylline increased cAMP and cAMP-dependent protein kinase activity ratio with an increase in cGMP levels which do not reach a statistically significant level.

The accelerating effect of pentoxifylline on relaxation in the perfused rat heart under our experimental conditions can be related to activation of the protein kinase system by an increase of the intracellular cAMP.

1 This work was supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas and the Comisión de Investigaciones Científicas. Buenos Aires, Argentina.
2 We thank Hoechst Laboratories for the partial support and Mrs Alicia R. Ramirez and María Prinzo for the excellent technical assistance.
3 R.W. Tsien, in: Advances in Cyclic Nucleotide Research, vol. 8, p. 363. Raven Press, New York 1977.
4 S.L. Keely and J.D. Corbin, Am. J. Physiol. *2*, 269 (1977).
5 M.M. Appleman, W.J. Thompson and T.R.ssell, in: Advances in Cyclic Nucleotide Research, vol. 3, p. 65. Raven Press, New York 1973.
6 W.R. Kukovetz and G. Pöch, in: Advances in Cyclic Nucleotide Research, vol. 1, p. 262. Raven Press, New York 1972.
7 J.E. Kuo, M. Shoji, N.L. Brackett and D.M. Helfmann, J. cyclic Nucleot. Res. *4*, 463 (1978).
8 P.V. Sulakhe, N.L. Leung and P.J. St. Louis, Can J. Biochem. *54*, 438 (1976).
9 J.T. Stull and J.E. Buss, J. biol. Chem. *252*, 851 (1977).
10 M. Tada, M.A. Kirchberger and A.M. Katz, J. biol. Chem. *250*, 2640 (1975).
11 M.L. Entman, G.S. Levey and S. Epstein, Circulation Res. *25*, 429 (1968).
12 M. Morad and E.L. Rolett, J. Physiol., London *224*, 537 (1972).
13 A.O. Grassi de Gende, A.D. Pérez Alzueta and H.E. Cingolani, Am. J. Physiol. *233*, 404 (1977).
14 H. Nawrath, Nature *262*, 509 (1976).
15 W.J. George, R.D. Wilkerson and P.J. Kadowitz, J. Pharmac. exp. Ther. *184*, 228 (1973).
16 T.W. Rall and T.C. West, J. Pharmac. exp. Ther. *139*, 269 (1963).
17 E.W. Sutherland, G.A. Robinson and R.W. Butcher, Circulation *37*, 279 (1968).
18 J.R. Blinks, C.B. Olson, B.R. Jewell and P. Braveny, Circulation Res. *30*, 367 (1972).
19 P.B. Henry, J.G. Dobson and B.E. Sobel, Circulation Res. *36*, 392 (1975).
20 E.G. Krause and A. Wollenberger, in: Cyclic Nucleotides: Mechanisms of Action, chapter 13, p. 229. Wiley, New York 1976.
21 J. Häggendal, Acta physiol. scand. *59*, 242 (1963).
22 W.B. Wastila, J.T. Stull, S.E. Mayer and D.A. Walsh, J. biol. Chem. *246*, 1996 (1971).
23 J.D. Corbin and E.M. Reimann, in: Methods in Enzymology, vol. 38, p. 287. Academic Press, New York 1974.
24 A.L. Steiner, Ch.W. Parker and D.M. Kipnis, J. biol. Chem. *247*, 1106 (1972).
25 G. Brooker, J. cyclic Nucleot. Res. *3*, 407 (1977).
26 J. Diamond, R.E. Ten Eick and A.J. Trapani, Biochem. biophys. Res. Commun. *79*, 912 (1977).
27 A.L. Steiner, A.S. Pagliara, L.R. Caase and D.M. Kipnis, J. biol. Chem. *247*, 1114 (1972).
28 C.J. Wiggers and L.N. Katz, Am. J. Physiol. *58*, 439 (1922).
29 W.W. Parmley and E.H. Sonnenblick, Am. J. Physiol. *216*, 1084 (1969).
30 E. Shinebourne and R. White, Cardiovasc. Res. *4*, 194 (1970).

The involvement of porphyrogenic steroids in the development of experimental porphyria[1]

V. Graef, S.W. Golf and G. Goerz

Institut für Klinische Chemie und Pathobiochemie der Universität Giessen, Friedrichstrasse 24, D-6300 Giessen (Federal Republic of Germany), and Universitäts-Hautklinik Düsseldorf, Moorenstrasse 5, D-4000 Düsseldorf 1 (Federal Republic of Germany), 6 December 1979

Summary. Hexachlorobenzene alters the hepatic steroid metabolism, and it was suggested that a porphyria was induced by overproduction of 5β-H-steroids. Structurally similar chlorinated hydrocarbons (pentachlorobenzene, pentachlorophenol, 2,4,5-trichlorophenol) without porphyrogenic activity did not affect the steroid metabolism in rat liver.

A great number of substances are able to provoke a hepatic porphyria in men and laboratory animals[2]. Furthermore, it was found that the ratio of 5β/5α-steroids in urine was elevated in man after administration of the porphyrogenic drug phenobarbital[3]. It was concluded that the activity of the hepatic NADPH: Δ^4-3-oxosteroid-5α-reductase was inhibited or diminished following administration of this drug. The decrease of NADPH: Δ^4-3-oxosteroid-5α-reductase ac-

Effect of 2,4,5-trichlorophenol (TCP), pentachlorophenol (PCP) and pentachlorobenzene (PCB) on steroid metabolizing enzymes in rat liver microsomes

Enzyme	Substrate	Control (nmoles/min · mg)	TCP (nmoles/min · mg)	PCP (nmoles/min · mg)	PCB (nmoles/min · mg)
NADPH: Δ^4-3-oxosteroid-5α-reductase	Test	34.11 ± 7.40	33.98 ± 2.22	32.46 ± 6.86	21.53 ± 1.55*
NADH: Δ^4-3-oxosteroid-5α-reductase	Test	35.59 ± 8.08	40.40 ± 4.62	39.21 ± 11.64	30.74 ± 4.10
NADPH: 3α-hydroxysteroid dehydrogenase	5α-DHT	4.71 ± 1.35	3.56 ± 0.42	4.88 ± 1.70	3.87 ± 0.20
NADH: 3α-hydroxysteroid dehydrogenase	5α-DHT	5.65 ± 0.81	6.08 ± 1.69	6.43 ± 0.34	6.41 ± 1.13
NADPH: 3β-hydroxysteroid dehydrogenase	5α-DHT	1.03 ± 0.57	0.82 ± 0.25	0.82 ± 0.12	0.80 ± 0.15
NADH: 3β-hydroxysteroid dehydrogenase	5α-DHT	1.18 ± 0.22	0.94 ± 0.22	1.30 ± 0.33	1.32 ± 0.36
NADPH: 3α-hydroxysteroid dehydrogenase	5β-DHT	10.25 ± 2.28	11.05 ± 0.99	11.61 ± 1.30	9.52 ± 0.39
NADH: 3α-hydroxysteroid dehydrogenase	5β-DHT	15.93 ± 3.70	16.92 ± 2.63	16.93 ± 1.66	14.42 ± 0.64
NADPH: 3β-hydroxysteroid dehydrogenase	5β-DHT	3.24 ± 0.81	4.01 ± 0.30	4.04 ± 0.53	2.90 ± 0.08
NADH: 3β-hydroxysteroid dehydrogenase	5β-DHT	6.16 ± 1.35	5.70 ± 0.71	7.02 ± 0.35	5.05 ± 0.24

Substrates: Testosterone (Test), 5α-dihydrotestosterone (5α-DHT), 5β-dihydrotestosterone (5β-DHT). * $0.005 < p < 0.01$; for all other values: $p > 0.05$.

tivity was confirmed by in vivo experiments[3] with the assumption that more 5β-steroids would be produced after inhibition of the NADPH: Δ^4-3-oxosteroid-5α-reductase. Another potent porphyrogenic substance is hexachlorobenzene. It was found in this laboratory that in the liver of rats which were fed with a diet containing 0.05% hexachlorobenzene the activities of NADPH: Δ^4-3-oxosteroid-5α-reductase and of all 3-hydroxysteroid dehydrogenases were significantly decreased, whereas the cytoplasmatic 5β-reductase was elevated[4]. We concluded from these findings that the changes in steroid metabolism would lead to an increased formation of unconjugated 5β-steroids (porphyrogenic steroids) which induce the porphyria. The aim of the present study was to investigate whether structurally similar chlorinated hydrocarbons without porphyrogenic activity (pentachlorobenzene, pentachlorophenol and 2,4,5-trichlorophenol) influence the activities of the steroid metabolizing enzymes in rat liver microsomes. As previously reported, the urine of rats which were fed with a diet containing pentachlorobenzene, pentachlorophenol and trichlorophenol, respectively, contained no increased amounts of total porphyrins, porphobilinogen and δ-aminolevulinic acid as compared with control animals[5].

Material and methods. Female Wistar rats (initial weight about 200 g) were fed over a period of 60 days with a diet containing 0.05% pentachlorobenzene, pentachlorophenol and 2,4,5-trichlorophenol, respectively, and water ad libitum. 4 animals for each group and 4 control animals which received a normal diet were then sacrificed. The livers were removed and microsomes were prepared as described previously[6]. Activities of NADPH: Δ^4-3-oxosteroid-5α-reductase, NADH: Δ^4-3-oxosteroid-5α-reductase and 3-hydroxysteroid dehydrogenases were determined as described previously[4]. The products were determined by gas chromatography. Statistical analysis was performed using Student's t-test.

Results and discussion. In liver microsomes of rats which were treated with pentachlorophenol and trichlorophenol the specific activities of NADPH: Δ^4-3-oxosteroid-5α-reductase, NADH: Δ^4-3-oxosteroid-5α-reductase and the 3-hydroxysteroid dehydrogenases were unchanged as compared with controls (table). In liver microsomes of rats which were fed with a pentachlorobenzene-containing diet, only the activity of NADPH: Δ^4-3-oxosteroid-5α-reductase was diminished. All other activities of the steroid metabolizing enzymes showed no significant alterations as compared with the controls (table).

These results confirm our assumption that the administration of porphyrogenic substances alters the activities of steroid metabolizing enzymes. This leads to an overproduction of 5β-steroids which are known as inducers of δ-aminolevulinic acid synthase, the rate limiting step in heme biosynthesis. Pentachlorophenol and trichlorophenol did not affect the 5α-reductases and the 3-hydroxysteroid dehydrogenases and no porphyria was observed[5]. After administration of pentachlorobenzene only NADPH: Δ^4-3-oxosteroid-5α-reductase was diminished, however; NADH: Δ^4-oxosteroid-5α-reductase and the 3-hydroxysteroid dehydrogenases were unchanged. It is possible that in this case more 5β-steroids may be produced owing to the deficiency of NADPH: Δ^4-3-oxosteroid-5α-reductase; however, since the 3-hydroxysteroid dehydrogenases are unchanged, testosterone is metabolized to 3-hydroxy-5β-androstan-(17-ol or 17-one) which is glucuronidated. The microsomal glucuronyltransferase was significantly induced by pentachlorobenzene (0.0398 ± 0.0147 nmoles/min · mg compared to 0.017 ± 0.0055 nmoles/min · mg for the control; $p < 0.025$), so that all 5β-steroids can be immediately transformed to glucuronides. It is known that the glucuronides of 5β-steroids have no porphyrogenic activity[7].

1 Dedicated to Prof. Dr L. Róka on the occasion of his 60th birthday.

2 G. Goerz, in: Dermatologie in Praxis und Klinik, vol. III, p. 38, 48–69. Ed. G.K. Korting. Thieme, Stuttgart 1979.
3 A. Kappas, M.L. Bradlow, D.R. Bickers and A.P. Alvares, J. clin. Invest. *59*, 159 (1977).
4 V. Graef, S.W. Golf and G. Goerz, Arch. Tox. *43*, 115 (1979).
5 G. Goerz, W. Vizethum, K. Bolsen, T. Krieg and R. Lissner, Arch. derm. Res. *263*, 189 (1978).
6 S.W. Golf, V. Graef and Hj. Staudinger, Hoppe-Seylers Z. physiol. Chem. *355*, 1499 (1974).
7 S. Granick and A. Kappas, J. biol. Chem. *242*, 4587 (1967).