Résumé. Nous avons préparé des anticorps contre l'hormone de mue β-ecdysone par immunisation avec un conjugué haptène-protéine. Les antisérums ont pu détecter des quantités de β-ecdysone aussi petites que 80 picogrammes. Les affinités relatives pour le β-ecdysone et l'α-ecdysone des antisérums sont de 20 contre 1.

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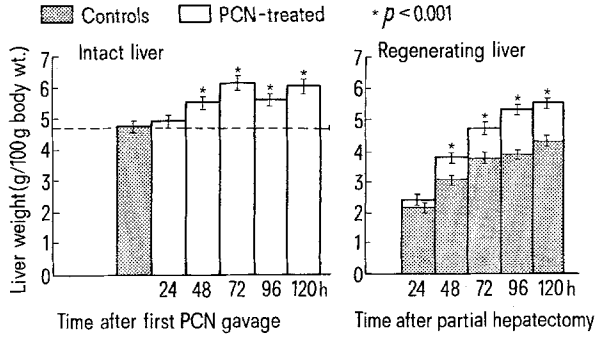
Effect of Pregnenolone-16 α-Carbonitrile on Mitotic Activity in the Intact and Regenerating Rat Liver

A number of investigators have noted mitotic activation of liver cells in animals treated with a variety of hepatic microsomal enzyme inducers[1–6]. But generally, the mitotic response was smaller than in partially hepatectomized rats. Because of this, the hepatomegaly following administration of some drugs was rather considered as hypertrophy of the parenchymal liver cells provoked by increased cell proteins, RNA and lipid content[7,8]. Nevertheless, the proliferative effect of some inducers under certain experimental conditions was high enough to be considered as a liver growth promoting factor[1,3,4,6]. However, because of the limited number of observations, the relationship between the proliferative effcet of the drugs and the induction of drug metabolizing enzymes is poorly understood.

Several groups have examined the response of regenerating liver to a variety of drug-metabolizing enzyme inducers during different stages of liver regeneration[9–14]. The general conclusion was that the metabolic adjustment of the liver imposed by partial hepatectomy exerts suppressive influence on the initial activation of the drug-metabolizing enzymes[9]. A recent report from this laboratory indicates that pregnenolone-16α-carbonitrile (PCN) represents an exception. The administration of a single or repeated doses of PCN, at time of partial hepatectomy or 20 h later, resulted in a 3- to 5-fold increase in ethylmorphine demethylase (EMD) and aniline hydroxylase (AH) activity in regenerating and sham-operated rat's livers[15]. Otherwise PCN, like other drug-metabolizing enzyme inducers, brings about a complex biochemical and structural reorganisation of hepatocytes accompanied by marked hepatomegaly[16–18]. In view of these data, it seemed of interest to examine the liver growth promoting influence of PCN in intact and partialy hepatectomized rats with special reference to mitotic activation of parenchymal liver cells.

Materials and methods. 2 series of experiments were performed on female ARS Sprague-Dawley rats (Madison, Wisconsin, USA), averaging 100 g (90–110 g) and maintained ad libitum on Purina Laboratory Chow (Ralston Purina Co. of Canada) and tap water. In the 1st series, we used 36 animals, of which 6 served as controls. In the 2nd, 60 partially hepatectomized rats[19] were divided into 2 equal groups. Here, the first group served as controls.

PCN (3β-Hydroxy-20-oxopregn-5-ene-16α-carbonitrile; Upjohn-U.14.975) was given to each remaining group in both series at the dose of 10 mg in 1 ml water (homogenized with a trace of Tween 80) twice daily by stomach tube for 1, 2, 3, 4, or 5 days. Treatment and partial hepatectomy were started at the same time. The animals were operated on and killed between 09.00 and 10.00 h. Liver sections were fixed in alcohol formol, embedded in paraffin and stained with hematoxylin-phloxine. The number of mitoses and parenchymal liver cells were determined by examining 115 visual fields at $\times 560$. The mitotic index



The effect of PCN on liver weight in intact and partially hepatectomized rats.

[1] M. JAPUNDŽIĆ, B. KNEŽEVIĆ, V. DJORDJEVIĆ-CAMBA and I. JAPUNDŽIĆ, Expl Cell Res. *48*, 163 (1967).
[2] T. S. ARGYRIS, J. Pharmac. exp. Ther. *164*, 405 (1968).
[3] T. S. ARGYRIS and D. R. MAGNUS, Devel. Biol. *17*, 187 (1968).
[4] T. S. ARGYRIS and D. L. LAYMAN, Cancer Res. *29*, 549 (1969).
[5] R. SCHULTE-HERMANN, R. THOM, I. SCHLICHT and W. KORANSKY, Arch. Pharmac. exp. Pathol. *261*, 385 (1968).
[6] E. HORVATH, A. SOMOGYI and K. KOVACS, Klin. Wschr. *48*, 385 (1970).
[7] H. REMMER and H. J. MERKER, Science *142*, 1657 (1963).
[8] W. STAUBLI, R. HESS and E. R. WEIBEL, J. Cell Biol. *42*, 92 (1969).
[9] J. HILTON and A. C. SARTORELLI, Adv. Enzyme Regul. *8*, 153 (1970).
[10] E. CHIESARA, F. CLEMENTI, F. CONTI and J. MELDOLESI, Lab. Invest. *16*, 254 (1967).
[11] T. E. GRAM, A. M. GUARINO, F. E. GREENE, P. L. GIGON and J. R. GILLETTE, Biochem. Pharmac. *17*, 1769 (1968).
[12] E. CHIESARA, F. CONTI and J. MELDOLESI, Lab. Invest. *22*, 329 (1970).
[13] J. HILTON and A. C. SARTORELLI, J. biol. Chem. *245*, 4187 (1970).
[14] R. SCHULTE-HERMANN, 5th intern. Congr. of Pharm. Ass. 1972, p. 206.
[15] I. JAPUNDŽIĆ, M. JAPUNDŽIĆ and S. SZABO, Eur. J. Biochem., *43*, 79 (1974).
[16] H. SELYE, *Hormones and Resistance* (Springer-Verlag, Heidelberg 1971).
[17] B. SOLYMOSS, J. WERRINGLOER and S. TOTH, Steroids *17*, 427 (1971).
[18] B. D. GARG, K. KOVACS, J. A. BLASCHECK and H. SELYE, J. Pharm. Pharmac. *22*, 872 (1970).
[19] H. WAELSCH and H. SELYE, Arch. exp. Path. Pharmak. *165*, 219 (1932).

Influence of pregnenolone-16α-carbonitrile on mitotic activity in the intact and regenerating rat liver

A) Intact livers	Hours after initiation of PCN treatment				
	Controls	24	48	72	96	120
	2.3 ± 0.44[a]	10.0 ± 1.49	11.86 ± 2.07	4.42 ± 0.47	1.40 ± 0.17	0.81 ± 0.06
	Significance (p)	<0.005[b]	<0.005[b]	<0.01[b]	>0.05[b]	<0.01[b]

B) Regenerating livers	Hours after operation and initiation of PCN treatment				
	24	48	72	96	120
Controls	14.61 ± 2.03	6.05 ± 0.40	3.55 ± 3.42	4.91 ± 0.98	3.65 ± 0.44
PCN-treated	19.33 ± 2.20	7.81 ± 0.81	6.73 ± 0.84	6.35 ± 0.45	5.25 ± 0.70
Significance (p)	>0.05[c]	>0.05[c]	<0.01[c]	>0.05[c]	>0.05[c]

[a] Mean (number of mitoses/1000 hepatocytes) of 6 rats ± standard error. [b] Compared with intact controls. [c] Compared with corresponding partially hepatectomized water treated controls.

(mitoses per 1000 hepatocytes) was then calculated. Statistical analyse of the data was performed according to t-test linear regression.

Results. The Figure shows a progressive increase in liver weight in intact (up to 3rd day) and in partially hepatectomized PCN-treated rats (up to 5th day).

PCN markedly increased mitosis in the hepatocytes of intact rats (Table). 24 h after initiation of treatment, the mitotic index in the liver of these animals was about 4 times higher than that of the controls. The proliferative activity of the hepatocytes, after reaching a peak at 48 h, returned to control values at 96 h. Mitotic activity was considerably stimulated by PCN treatment in the liver of partially hepatectomized rats also. However, this change was statistically significant only at the 72-h stage.

PCN produced cellular enlargement, cytoplasmic vacuolization and a decrease of PAS-positive material. No pathological changes could be detected in the hepatocytes. The mitoses did not show any anomalies, and all phases of cell division were evident.

The mitotic figures in the liver of intact and hepatectomized PCN-treated rats were localized mainly in the peripherial areas of the lobules.

Discussion. Earlier studies[16–18, 20] have shown that PCN increases hepatic weight, microsomal proteins, NADPH-cytochrome c-reductase activity and cytochrome P-450 content. The present study provides evidence that this steroid markedly enhances mitotic activity in intact and regenerating liver tissue. The significant increase of mitotic activity, already 24 h after administration of PCN, indicates that DNA synthesis in the liver begins within a few hours after the first gavage, since it in known that S and G phases last for about 20 h[21].

The induction or regression of hepatic drug-metabolizing enzymes activity is always associated with changes in liver weight and total protein content[3, 4, 22]. DNA and nuclear count are also increased in the liver of rats treated with different inducers[22]. However, the contribution of hepatocyte proliferation to an increase in liver wet weight was never as high as in PCN-treated animals.

The competitive relationship was reported between the regenerative liver growth and the induction of drug-metabolizing enzymes[9]. It was shown that the induction of drug-metabolizing enzyme is lower or completely delayed in regenerating liver of rats treated with different enzyme inducers[9]. Based on these data it was suggested that the lessened response of regenerating liver to inducers is related to inability of the replicating hepatocytes to participate simultaneously in the induction of the microsomal enzymes[9]. The results with PCN represents an exception. PCN stimulates regenerative and intact liver growth and at the same time it induces manifold aniline hydroxylase and ethylmorphyne demethylase activity[15]. These data could be taken as indicating that replicating hepatocytes are able to participate in the transcriptional processes, but only if DNA synthesis and the enzyme induction take place in the same population or cells. However, this seems unlikely. Some evidence indicate that there is difference in spatial distribution between dividing and drug-metabolizing hepatocytes. We have noticed that mitotic figures in the intact and regenerating liver of rats treated with PCN were localized mainly in the peripherial areas of the lobules, which is in accordance with the results of GRISHAM[23]. On the other hand, BRODIE[24] has shown recently that centrilobular cells contained high concentration of drug-metabolizing enzymes and that smooth endoplasmic reticulum containing the enzymes is localized mainly in the central zone of liver lobules. If two cell types participate in DNA synthesis and enzyme induction, the competition between the two processes is excluded at least at subcellular level.

Résumé. Le prégnénolone-16α-carbonitrile (PCN) provoque l'hypertrophie et l'hyperplasie du foie chez les rats intacts et à un moindre degré chez les rats partiellement hépatectomisés. Le nombre augmenté des mitoses des hépatocytes joue un rôle important dans l'hépatomégalie provoquée par le PCN.

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[20] B. D. GARG, K. KOVACS and B. TUCHWEBER, Virchows Arch. part B Zellpath., in press.
[21] E. STÖCKER and W. D. HEINE, Beit. Path. Anat. *131*, 410 (1965)
[22] T. S. ARGYRIS, Devel. Biol. *25*, 293 (1971).
[23] J. W. GRISHAM, Cancer. Res. *22*, 842 (1962).
[24] B. B. BRODIE, A. K. CHO, G. KRISHNA and M. D. REID, Ann. N.Y. Acad. Sci. *179*, 11 (1971).
[25] The authors wish to thank the Upjohn Company, Kalamazoo (Michigan, USA) for the PCN used in this study.