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Summary

One time i.p. injections of 5 - 20 mg Pregnenolone-16α-carbonitrile (PCN) effects the mitotic activity of rat liver cells during a time period of 24 hours to be 20 times higher than the normal mitotic rate. This, however, does not result in an measurable increase of cell numbers and cell volumes. Furthermore, total liver DNA, RNA, protein and dry-weight remain unchanged.
The injection of 7 times 10 mg PCN also results in the elevation of the mitotic rate. Return of the elevated mitotic rate to base level takes 18 days from the time the last injection of PCN was administered. During this time the number and the average volume of liver cells increases by 28 % and 30 %, respectively. The dry weight of liver and tetraploid nuclei increase in number, whereas RNA- and protein content remains unchanged. All parameters have reached the base line after 4 - 6 weeks following the last injection of PCN.
No histological changes were observed after multiple doses of PCN that cause hyperplasia and hypertrophy of liver tissue. Hypertrophy of liver cells follows the observed hyperplasia and therefore is not expected to be the cause of the hyperplastic processes.

Zusammenfassung

Nach einmaliger i.p. Gabe von 5 - 20 mg Pregnenolon-16α-carbonitril (PCN) an Ratten steigt die mitotische Aktivität der Hepatozyten innerhalb von 24 Stunden bis zum 20-fachen der normalen Mitoserate an; dies führt aber nicht zu einer meßbaren Zunahme der Zellzahl und des Zellvolumens. Außerdem bleiben die Gesamt DNS, RNS, Protein oder Trockengewicht der Leber unverändert. Nach siebenmaliger Gabe von 10 mg PCN steigt die Mitoserate ebenfalls an. Die Normalisierung der Mitoserate dauert bis 18 Tage nach der letzten PCN-Gabe. In diesem Zeitraum erhöht sich die Zahl und das mittlere Volumen der Leberzellen um 28 % bzw. 30 %. Außerdem nehmen das zelluläre Trockengewicht und die tetraploiden Zellkerne zu, während der RNS- und Proteingehalt der Zellen unverändert bleiben. 4 - 6 Wochen nach der letzten PCN-Gabe haben sich alle Parameter wieder weitgehend normalisiert. Die Hyperplasie und Hypertrophie des Lebergewebes nach mehrmaliger PCN-Gabe führt zu keiner histoligisch nachweisbaren Veränderung im Aufbau des Lebergewebes. Die Hypertrophie der Leberzellen tritt zeitlich nach der Hyperplasiephase auf, und kann deshalb nicht als Ursache für die Auslösung der hyperplastischen Vorgänge angesehen werden.

Key-Words: Pregnenolone-16α-carbonitrile – Liver cells – Hyperplasia – Hypertrophy

Pregnenolone-16α-carbonitrile (PCN) is known to exert a catatoxic effect on animals poisoned with digitoxin, hexobarbitone, zoxazolamine, nicotin, chlordiazepoxide, colchicine and others (5, 12, 13, 14). From this, importance for human chemotherapy could develop, if no major side effects would be observed. This may be of importance concerning a single high dosage in cases of acute poisoning or several low doses in cases of chronic poisoning.

Considering side effects, it is of importance to study the effect of PCN on liver, since this is the organ where detoxification takes place.

Initial experiments could show, that the liver increased in size after application of multiple doses of PCN. In order to find out, whether this increase is due to hypertrophy or hyperplasia and whether this is a process limited to the liver, we determined physical and chemical parameters of rat liver cells after one and seven injections of PCN. We measured wet and dry weights of liver, mean cell and nuclei volumes during a period of 180 days. Changes in ploidy pattern of cells, DNA and RNA were obtained, as well as mitotic rates of liver cells and the number of cells, containing two nuclei were counted from histological preparations.

Materials and Methods

In these experiments female rats from the BDE and Wistar strains, weighing 102 ± 7 g were used. The animals were purchased from the Zentralinstitut für Versuchstierkunde (Hannover) and from the Institut für Strahlen- und Umweltforschung (Neuherberg/München). The animals were kept in cages at 23°C with food (Altromin) and water ad libitum. Injections were given i.p. once with a dose of 5, 10 and 20 mg PCN, or seven times the dose of 10 mg PCN in 1 ml of 0.9 % saline. The suspension was prepared by sonification. The animals were injected either at 6 AM ± 15 minutes or at 6 PM. Animals were sacrificed at 6 AM ± 20 minutes after the last injection in intervals. Thereafter they were bled under ether anesthesia by aspirating blood from the aorta. The rat livers were perfused with 20 ml of 300 mosm Hanks medium (pH 7.4) kept at 0°C, that contained 0.05 % collagenase and 0.1 % hyaluronidase (Boehringer, Mannheim). For histological preparations a piece of the lobus caudatus was prepared according to the method of *Davidson* (11). The sections of 5 µm were stained with Haematoxylin-Eosin. The mitotic rate was assessed by counting 10.000 cells per section. The weight of the perfused liver was determined. Approximately 1 g of liver tissue was used to separate nuclei on sucrose (3, 18, 19). From the remaining liver, isolation of single liver cells was performed, according to the method of *Howard* and *Pesch*, modified by *Tongendorff* (6, 16). Vitality of the cells was established by staining with Trypan blue (1). Measurement of the mean cell and nuclei volumes was carried out as described by *Coulter*, modified by *Kachel* et al. (7, 8). The apparatus was balanced by electric means, and the date obtained for measurements of cells and nuclei, respectively, were those shown in the table.

	cells	nuclei
Diameter of orifice	200 µm	100 µm
Length of orifice	126 µm	75 µm
Medium	phosphate buffer pH 7.4	Tris HCl buffer pH 7.4
Temperature	24°C	24°C
Current	0.3 mA	0.3 mA
Resistance	61 Ωcm	136 Ωcm

Rats of another experiment were used to determine liver dry weight, total DNA, RNA and protein values. The animals were bled in the manner described, livers were excised and homogenized, taking 1 part of liver mixed with 4 parts of destilled water, by 12 strokes at 15.000 rpm in an Elvehjem-Potter fitted with a Teflon pistil. The homogenate was further filtered through a plastic sieve with a meshwidth of 0.15 mm.

To determine the dry weight of liver before weighing 10 % of the homogenate was dried at 105°C. The preparation of DNA was modified according to *Burton* (2). 500 µl of the homogenate were suspended in 5 ml of a 0.5 M PCA-solution and kept at 4°C for 30 minutes. The mixture was centrifuged for 15 min. at 400 x g and the sediment was stirred in 0.5 ml of a 0.5 N NaOH solution at room temperature for 30 min. Another 4.5 ml of the 0.5 M PCA-solution were added and the mixture was kept at 4°C for 30 min. The sediment was obtained by centrifugation as described and resuspended in 5 ml of 0.5 M PCA and hydrolized for 20 min at 85°C. After centrifugation hydrolysis of the pellet was repeated and both supernatants combined. RNA determinations were carried out on 250 µl aliquots of the homogenate, according to the method described by *Ceriotti* (4). 250 µl of the homogenate were used to estimate total protein concentrations (9).

Results

Effect of a single injection of PCN. – (Table 1, Table 2, Table 3).
After a single i.p. injection of PCN the body weight of experimental animals does not change when compared to controls. Also, the liver weights, expressed in percentages of total body weight do not differ from those of controls. In contrast, the mitotic rate increases during a period of 24 hours in relation of different doses up to 10 to 20 times the base level (2 mitoses/ 10.000 cells). The base level is reached again 3 days after injection of PCN (Fig. 1). Under the present experimental conditions the average cell volume of hepatocytes indicated a slight increase in the upper range of the normal distribution of cell volumes, and was measured to be over 6800 μ^3. After approximately 25 days after injection, the low normal level is reached. The values are shown in Fig. 2. Fig. 3 shows, that the mean volume of nuclei increased about 25 % in a period of 24 hrs to 50 μ^3 with diploid nuclei and 100 μ^3 with tetraploid nuclei, and again decreased to base level in 15 days. There was no difference to control values concerning the dry weight, total protein content and RNA and DNA concentrations. The relation of diploid to tetraploid nuclei was observed to be 1:1.500 and 1:16.595 after 100 days and 180 days, respectively. That is in the normal range.

Table 1. Effect of PCN on rats and excised livers in comparison to untreated controls.

days	body weight (g) PCN before	PCN after	controls before	controls after	liver weight (g) PCN	liver weight (g) controls	dry weight mg/g liver PCN	dry weight mg/g liver controls	protein/g liver (mg) PCN	protein/g liver (mg) controls
1 x 10 mg PCN										
1	97 ± 2	103 ± 1,5	97 ± 2	102 ± 2	5,92 ± 0,68	5,56 ± 0,54	293 ± 4	298 ± 2	192 ± 10	176 ± 8
2	100 ± 0	103 ± 2	96 ± 1	98 ± 1	5,50 ± 0,10	5,01 ± 0,24	283 ± 0	282 ± 2	222 ± 8	222 ± 8
3	104 ± 1	118 ± 0	101 ± 1	115 ± 3	6,97 ± 0,01	6,43 ± 0,23	299 ± 1	300 ± 2	206 ± 3	170 ± 4
4	106 ± 3	121 ± 2	105 ± 3	121 ± 2	7,01 ± 0,01	6,88 ± 0,25	293 ± 2	291 ± 2	216 ± 12	236 ± 15
5	100 ± 2	114 ± 0	104 ± 4	114 ± 5	6,08 ± 0,09	6,49 ± 0,74	285 ± 2	300 ± 5	204 ± 3	192 ± 8
10	110 ± 1	148 ± 3	108 ± 1	145 ± 0	9,19 ± 0,10	8,32 ± 0,03	289 ± 2	285 ± 0	208 ± 0	198 ± 4
15	107 ± 1	157 ± 3	108 ± 1	155 ± 1	8,92 ± 0,81	8,61 ± 0,63	290 ± 2	293 ± 1	208 ± 15	200 ± 8
7 x 10 mg PCN										
2	98 ± 1	154 ± 4	108 ± 1	155 ± 1	13,05 ± 0,13	8,61 ± 0,63	279 ± 4	293 ± 1	160 ± 15	200 ± 8
10	104 ± 1	181 ± 1	101 ± 1	176 ± 0	12,98 ± 1,02	10,79 ± 0,34	296 ± 12	297 ± 5	192 ± 12	198 ± 8
18	98 ± 1	193 ± 5	100 ± 6	194 ± 2	11,79 ± 0,30	10,56 ± 0,52	316 ± 4	312 ± 5	204 ± 12	188 ± 0

Table 2. Effect of PCN on rats and excised livers in comparison to untreated controls.

days	DNA/g liver (mg) PCN	DNA/g liver (mg) controls	RNA/g liver (mg) PCN	RNA/g liver (mg) controls
1 x 10 mg PCN				
1	3,94 ± 0,6	3,89 ± 0,3	20,8 ± 0,4	23,0 ± 0,0
2	4,51 ± 0,3	4,10 ± 0,0	19,6 ± 0,1	19,3 ± 0,2
3	4,59 ± 0,2	4,27 ± 0,1	21,2 ± 0,4	22,9 ± 0,2
4	—	—	16,1 ± 0,8	18,5 ± 1,5
5	4,22 ± 0,2	4,23 ± 0,1	17,5 ± 0,2	17,8 ± 0,7
10	—	—	20,4 ± 0,4	17,2 ± 0,4
15	3,55 ± 0,2	3,17 ± 0,1	17,3 ± 0,3	17,5 ± 0,6
7 x 10 mg PCN				
2	2,83 ± 0,2	3,17 ± 0,1	17,3 ± 0,3	17,5 ± 0,6
10	3,34 ± 0,2	3,47 ± 0,2	24,8 ± 1,2	24,8 ± 0,8
18	4,21 ± 0,2	4,57 ± 0,0	16,0 ± 0,5	15,8 ± 0,7

Table 3. Ratio of diploid and tetraploid nuclei.

days	diploid (%)	tetraploid (%) ± S.D.
1 x 10 mg PCN		
0	52,3	47,7 ± 2,9
20	40,5	59,5 ± 3,4
100	40,0	60,0 ± 2,5
180	37,6	62,4 ± 0,9
7 x 10 mg PCN		
0	52,3	47,7 ± 2,9
control 2	48,9	51,1 ± 1,5
2	42,5	57,5 ± 1,4
18	37,3	62,7 ± 0,6
100	37,0	63,0 ± 3,3
180	34,0	66,0 ± 1,3

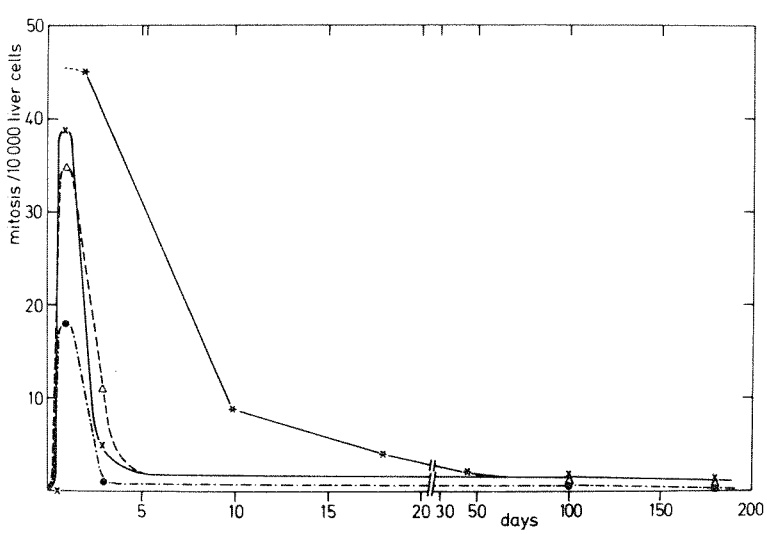


Fig. 1. Number of mitoses/10.000 liver cells after different doses of PCN.
Single doses · — ● — · 5 mg
— x — 10 mg
— — △ — — 20 mg
multiple doses — * — 7 x 10 mg
t_0 - first day after the last injection of PCN.

Fig. 2. The average volume of liver cells after different doses of PCN.
Single doses •—●—• 5 mg
—x— 10 mg
——Δ—— 20 mg
multiple doses —*— 7 x 10 mg
t_0 - see Fig. 1.

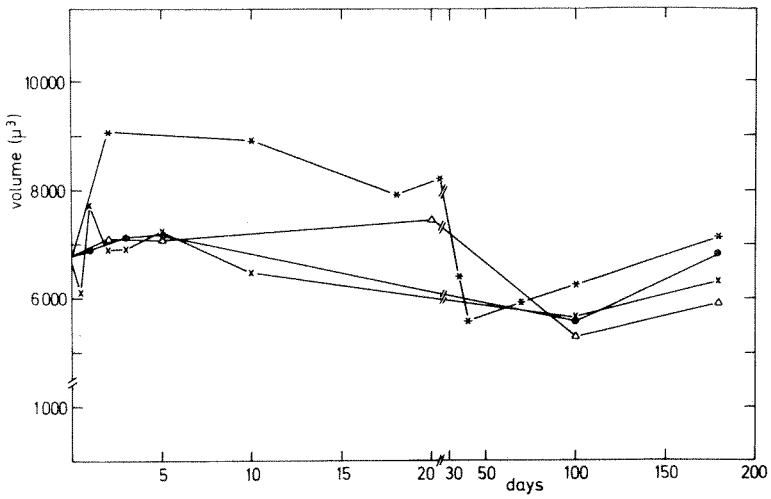


Fig. 3. The average volume of liver cell nuclei after one and seven injections of 10 mg PCN.
x 1 time injection
Δ 7 time injection
t_0 - see previous Figs.
The lower curves represent the data for diploid nuclei (a), the upper curves those for tetraploid nuclei (b).

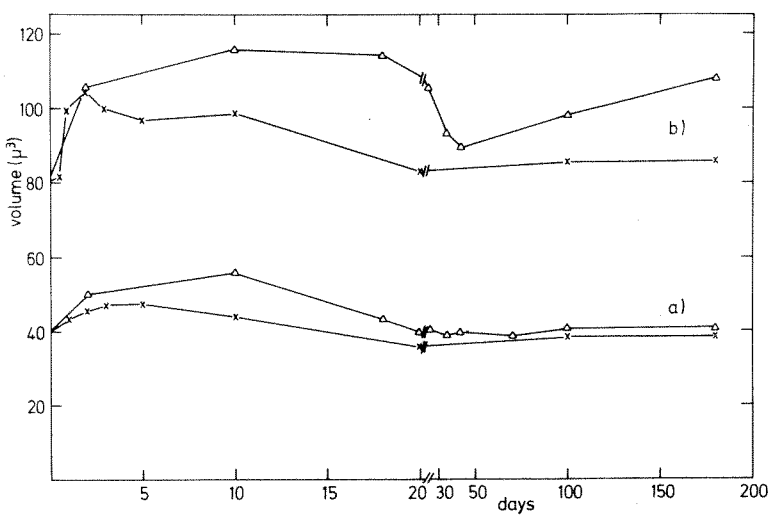
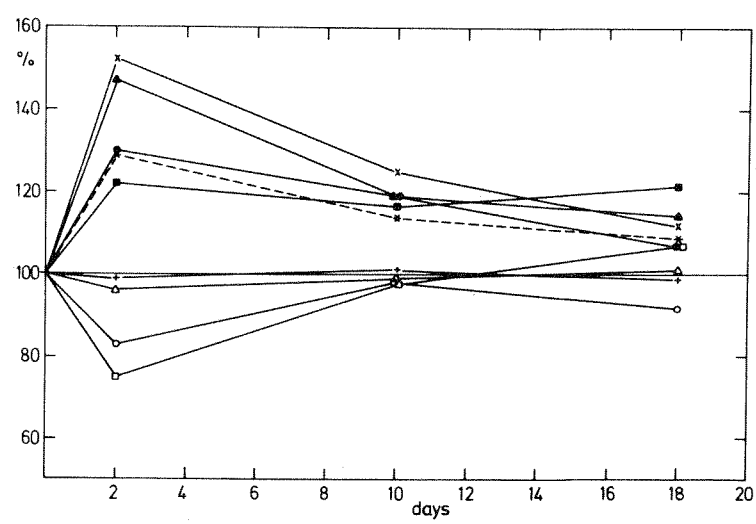


Fig. 4. Different parameters to characterize the effect of PCN (7 x 10 mg) on liver cells, whereby t_0 is the last injection day. In this experiment the data observed for controls were set at 100 %.
* average volume of liver cells
x liver weight
▲ total dry weight of liver
■ protein content of total liver
● DNA content of total liver. This value was obtained by substracting the DNA for formation of tetraploid cells and the DNA content estimated for the appearance of cells with 2 nuclei.
\+ total RNA values of liver
Δ dry weight/g liver
□ protein content/g liver
○ DNA content/g liver
The increase in DNA by 28 % accounts for the new formed diploid cells. The decreased protein concentration (□) corresponds to the observed hypertrophy of cells. This is also seen in the increase of the average cell volume (*).



Effect of multiple injections of PCN. – (Table 1, Table 2, Table 3).
After the injection of 10 mg PCN (7 times) in intervals, again no increase in total body weight was observed. However, the total liver weight was increased up to 50 % when compared to controls (Fig. 4). In a similar manner, the percentage of liver weights increased, when compared to total body weights. Fig. 4 also shows, that the absolute values for liver weights in experimental animals remained constant from the 15th day of the experiment, whereas the liver weights of control animals increased with increasing body weight. After 6 weeks from the beginning of the experiment, the livers stayed 13 % larger. After PCN treatment, the final liver weight appears to be reached earlier. The absolute values of the dry weight increased with increasing liver weights. Only the values for dry weight per g liver remained approximately 5 % under those of controls, but were observed to again reach control values during the time of the experiment. Fig. 1 shows, that the mitotic rate, 2 days after the last injection was increased 22 fold and reached the normal value after 2 weeks. The average volume for nuclei increased by approximately 30 %. This value remained for 3 weeks and reached control values during the 4th to 5th week (Fig. 3). Fig. 2 shows the average cell volume to increase by 30 % to 9000 μ^3. This remains constant for 2 weeks and decreases after 5 weeks to control levels. There is no significant increase in RNA values after seven injections of PCN. After 16 days, following the start of the experiment, the absolute values obtained for DNA were 36 % higher than those for controls, whereas the DNA values/g liver were about 10 % lower when compared to controls (Fig. 4). The protein content of total liver was approximately 22 % higher than that measured for controls, whereby the values per g liver decreased by 25 % (Fig. 4). DNA values as well as total protein concentrations were observed to adjust to control values during a period of 3 weeks. After the last injection of PCN the ratio of diploid to tetraploid nuclei changed in the following manner: for experimental animals the ratio was found to be 1:1.353, for controls a ratio of 1:1.045 was obtained. During a time period of 180 days the values did not quite reach those of controls but stayed slightly above. The values for days 100 and 180 were 1:1.703 and 1:1.942, respectively. The number of cells, containing 2 nuclei determined from histological slides were at the onset of the experiment 0.99 + 0.06 % (n = 4). No significant change was observed after a single dose or multiple injections of PCN during days 0 to 30. Also, no differences were found in results from comparable animals of either the BDE or Wistar strain.

Discussion

In the literature PCN is claimed to have a high catatoxic effect, when used on experimental animals (13). Also, it is known to result in increased enzyme activity of mitochondria as well as mitotic activity of liver cells (5). The total body weight of experimental animals was not different from that of controls. In order to obtain some idea on how PCN effects animals in short and longterm experiments, we considered the following parameters: total body weight, liver weight, mitosis, changes in the number of cells with 2 nuclei, mean cell volume, mean volumes of nuclei and changes in ploidy patterns of cells. In an independent experiment we evaluated the dry weight of liver, its DNA and RNA content and the total protein concentration. After a single dose of PCN, only the mitotic rate and the average volume of nuclei changed (Fig. 3). The significant increase in the mitotic rate (Fig. 1) is of too short a duration, to effect a measurable increase in the total number of liver cells. This is further confirmed by the constant value for liver DNA (Table 2). At present, there is no valid explanation for the increase of the average volumes of diploid and tetraploid nuclei. So far, however, it may be excluded, that this increase is due to an increased DNA synthesis, since total DNA did not increase during the experiment (Fig. 4).

After multiple doses of PCN (Fig. 4), all parameters differed from those of controls, with the exception of body weight, dry weights/g liver and RNA concentrations.

The increase in the mitotic rate and its duration over a period of time (Fig. 1), as well as the lack of dead cells in the histological preparations, is an indication for the appearance of many

new cells. This is confirmed by the increase in total liver DNA of 36 % (Fig. 4). This observation, however, is not conclusive evidence for the increase in cell numbers. This could also be explained by changes in ploidy of an already present cell nuclei as well as the presence of a larger number of cells with 2 nuclei would account for the increased cell numbers. According to the distribution of nuclei volumes, tetraploid cells increase by 6 % when compared to controls. In contrast, octoploid cells did not increase significantly. Since we could not observe a significant increase in the number of cells with 2 nuclei, the increase in DNA for the occurance of new nuclei was estimated to be 36 – 6 = 30 %, which can be attributed to the new formation of nuclei. This value is based on the estimation, that only already existant nuclei will transform to the tetraploid state. One percent of the new cells contain 2 nuclei. This can be shown in histological slides, where no changes in the numbers were observed. This, however, indicates that 2 x 1 = 2 % of the 30 % DNA is located in cells with 2 nuclei. Therefore, the actual increase in cell numbers is 30 – 2 = 28 %, which results in a definite hyperplasia of liver after administration of multiple doses of PCN.

In addition to the increase in the total number of liver cells the average cell volume increases by 30 %, whereas the protein concentration decreases by 25 % (Table 1). This could be explained by swelling of cells caused through high uptake of water. Since there is no change in RNA concentration and in the values for dry weight/g liver, this cell enlargement is not due to water uptake, but to the increase of nonproteinaceous material due to the effect of multiple PCN dosage. The total protein values per cell did not change, since the decrease by 25 % in protein concentration is balanced by the increase in the mean cell volume by 30 %. Liver weight increased by 50 % when compared to control animals. This can be explained by hyperplasia and hypertrophy of liver cells by 28 %, respectively. The question remains, whether hyperplasia is an independent occurance or a result of hypertrophy. Some of our recently published results indicate, that hypertrophy has no effect on hyperplasia in partially hepatectomized animals (17). Similar results were obtained after administration of PCN, where we could show that an increased mitotic rate was followed by hypertrophy (Fig. 1). Evidence was provided by *Paulini* et al. (10), that phenobarbital effects the correlation between hypertrophy and hyperplasia of liver cells. This, however, does not explain, whether this relation is basic or coincidental.

A c k n o w l e d g e m e n t

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References

1 *Bessis, H.:* Ciba Foundation Symposium in Cellular Injury ed. Renck, A.V. Side, J. Knight p. 287 Churchill London 1964
2 *Burton, K.:* A Study of the Conditions of the Mechanism of the Diphenylamine Reaction for the Colometric Estimation of Deoxyribonucleic Acid., Biochem. J. 62 (1956) 315
3 *Busch, H., J.C. Choi, E. Daskal, A. Inagaki, M.O.J. Olson, R. Reddy, T.S. Ro-Coi, H. Shibata, L.C. Yeoman:* Isolation and composition of nuclei and nucleoli. 5th Karolinska Symposium on Research Methods in Reproductive Endocrinology, Gen Transcription in Reprodutive Tissue. (1972) p 35
4 *Ceriotti, G.:* Determination of Nucleic Acids in Animal Tissues. J. Biol. Chem. 214 (1955) 59
5 *Garg, B.D., K. Kovacs, B. Tuchweber, J.D. Khandeka:* Effect of Prgnenolone-16α-carbonitrile, a Microsomal Enzyme Inducer, on Regenerating Rat-Liver. Act. Anatom 91 (1975) 161
6 *Howard, R.B., J.C. Lee, L.A. Pesch:* The fine Structure, Potassium Content, and Respiratory Activity of Isolated Rat-Liver Parenchymal Cells Prepared by Improved enzymatic Techniques. J. Cell. Biol. 57 (1973) 642
7 *Kachel, V.:* Methoden zur Analyse und Korrektur apparativ bedingter Meßfehler beim elektrischen Verfahren zur Teilchengrößenbestimmung nach Coulter Diss. Berlin 1972
8 *Metzger, H., G. Valet, V. Kachel, G. Ruhenstroth-Bauer:* The Calibration by electronic means of absolute Volume of particles. Blut 25 (1972) 179

9 *Lowry, O.H., N.J. Rosebrough, A.L. Farr, R.J. Randall:* Protein measurement with the Folin Reagent. J. Biol. Chem. 193 (1951) 265
10 *Paulini, K., K. Grimmel, G. Beneke:* Anwendbarkeit der "Critical-Maß"-Theorie für die phenobarbitalinduzierte Lebervergrößerung. Beitr. Path. 142 (1971) 129
11 *Pera, F.:* Heterochromatin, repetetive DNS und Nucleolen in Leberzellen von Microtus agrestis Histochem. 30 (1972) 82
12 *Romeis, B.:* Mikroskopische Technik. 16. Aufl. Oldenbourg, Verlag München-Wien 1968
13 *Selye, H.:* Pharmaco-chemical interrelations among catatoxic steroids. Rev. Can. Biol. 29 (1970) 49
14 *Solymoss, B., J. Werringloer, S. Toth:* The Influence of Pregnenolone-16α-carbonitrile on Hepatic mixedfunction Oxygenase. Steroids 17 (1971) 427
15 *Szabo, S., H. Selye, P. Kourounakis, Y. Taché:* Comparative Studies on the Effect of Adrenocorticotrophic Hormone (ACTH) and Pregnenolon-16α-carbonitrile (PCN) upon Drug Response and Distribution in Rats. Biochem. Pharm. 23 (1974) 2083
16 *Tongendorff, J.:* Versuche zur Proliferations- und Volumenregulation der Hepatozyten nach experimenteller Teilhepatektomie. Dissertation 1973, Univ. München
17 *Tongendorff, J., R. Trebin, G. Ruhenstroth-Bauer:* Critique of the "Critcal Mass" Hypothesis of the Regeneration of Liver Cells. Am. J. Path. 80, (1975) 519
18 *Valet, G., S. Silz, H. Metzger, G. Ruhenstroth-Bauer:* Electrical sizing of liver cell nuclei by the particle beam method. Mean Volume, Volume Distribution and Electrical Resistance. Acta Hepato-Gastroent. 22 (1975) 274
19 *Widnell, C.C., J.R. Tata:* A procedure for the isolation of enzymically active rat-liver nuclei. Biochem. J. 92 (1964) 313

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Treatment of Extrahepatic Occlusive Jaundice with Activated Charcoal Hemoperfusion in Dogs

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Summary

To find the feasibility of treatment for congenital bile duct atresia, we studied the usefullness of extracorporeal hemoperfusion over activated charcoal in canine obstractive jaundice.

One, three and five weeks after ligation and disection of common bile duct in 5 dogs we performed the hemoperfusion over activated charcoal extracorporeally (group 3). In this animals we examined hematological and blood coagulation studies, serum electrolyte levels, kidney function tests and liver chemistries. As control in 5 animals we carried out after sham operation the perfusion without common bile duct ligation (group 2) and in 5 animals only common bile duct ligation without perfusion (group 1).

In the liver chemistries we found 2 weeks after 2nd and 3rd perfusion (5 and 7 weeks after bile duct ligation) lower levels of serum bilirubin, GOT, GPT and SDH in treated group than in non-treated jaundiced animals. It suggest the effectiveness of hemoperfusion with activated charcoal in the treatment of occlusive jaundice.

There were no alteration in the hematological studies, serum electrolyte levels and kidney function tests. PT and PTT was prolonged in the jaundiced animals there were no significant differences with and without hemoperfusion.

Zusammenfassung

Um die Behandlungsmöglichkeiten der kongenitalen Gallengangsatresie zu untersuchen, haben wir in dieser Arbeit versucht, einen Verschlußikterus beim Hund durch extrakorporale Hämoperfusion mit Aktivkohle zu beeinflussen.

Zu diesem Zweck haben wir eine, drei und fünf Wochen nach Ligatur und Durchtrennung des Gallenganges bei fünf Hunden die Hämoperfusion durchgeführt (Gruppe 3). Gleichzeitig untersuchten wir bei diesen Tieren Hämatologie, Blutgerinnung, Serumelektrolyte, Nierenfunktion und Leberchemie. Zur Kontrolle wurde bei 5 Hunden nach Scheinoperation ohne Ligatur des Gallenganges die Perfusion durchgeführt (Gruppe 2) und bei 5 Hunden nur der Gallengang ligiert (Gruppe 1).

Bezüglich der Leberchemiewerte waren Serumbilirubin, GOT, GPT und SDH 2 Wochen nach der zweiten und dritten Perfusion (5 bzw. 7 Wochen nach der Gallengangsligatur) deutlich niedriger bei den behandelten als bei den nicht perfundierten Tieren. Dies läßt auf die Wirksamkeit der Hämoperfusion mit Aktivkohle bei Verschlußikterus schließen.

Hämatologische Ergebnisse, Serumelektrolytsspiegel und Nierenfunktionstest blieben unbeeinflußt. PT und PTT waren bei den ikterischen Tieren verzögert; jedoch gab es keine signifikanten Unterschiede mit oder ohne Hämoperfusion.

Key-Words: Occlusive jaundice – Hemoperfusion over charcoal – Artificial liver

In 1964 *Yatzidis* (14) reported that extracorporeal hemoperfusion over activated charcoal was effective in removing drugs, uric acid, creatinin and some organic substances from patients with uremia and intoxication.

Thereafter the other authors confirmed these effects of charcoal in clinical (3) and experimental (6) studies.

In 1972 *Chang* (2) demonstrated the effect of microencapsulated charcoal hemoperfusion on a patient with hepatic coma. The bilirubin levels of serum fell and her state of consciousness improved.

Gazzard et al. (5) treated patients with acute liver failure in grade IV coma with extracorporeal charcoal hemoperfusion. The patients recovered consciousness within 1 to 7 days after the first period of perfusion.

Both experiences suggest that the activated charcoal absorbs coma substances.