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ALPHA-FETOPROTEIN AND SERUM HORMONE LEVELS FOLLOWING LIVER INTOXICATION WITH CARBON TETRACHLORIDE

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SUMMARY

Rats were submitted to carbon tetrachloride intoxication at two different doses. Serum level of estradiol, progesterone, cortisol and thyroxin were measured by radioimmunoassays and correlated with the histological evidences of liver regeneration and serum alpha-fetoprotein levels. A two fold increase of progesterone was observed 48hrs after CCl_4 administration. Cortisol levels were moderately increased at both doses of CCl_4. Despite the five fold increase of alpha-fetoprotein (which is the major estradiol binding protein in these sera) no changes in estradiol levels were observed. Thyroxin levels showed a two fold increase after 72hrs. This result contrasts with the drop of this hormone after partial hepatectomy which has been previously published. These experiments show that a new hormonal imbalance (directly or indirectly due to the toxic) is involved both in liver regeneration and alpha-fetoprotein synthesis.

INTRODUCTION

A previous work by Belanger et al (1) showed that several types of hormones are able to influence alpha-fetoprotein (AFP) synthesis. In new born rats, glucocorticoids and to a lesser extend thyroxin produced a decrease of AFP synthesis. Conversely, in animals submitted to AFP inducing conditions (CCl_4 intoxication or 3'-methyl-diaminobenzidine (3'DAB) induced liver tumors) corticoids increased the AFP level. A similar result was obtained by De Nechaud et al (2) on hepatoma 8994 in vitro . On the other hand, AFP, at least in mice and rats, is an estradiol binding globulin and the variations of its serum level may influence the level of sexual steroids (3,4) . Despite the interest in the role of hormones in liver regeneration (5) and AFP synthesis, no measurement of serum hormone levels have been made following intoxication with CCl_4 and subsequent liver regeneration and AFP synthesis. In the present work we measured simultaneous changes in the serum level of estradiol,

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progesterone, cortisol, thyroxin and AFP. In addition, variations of liver weight and histological modifications were studied in the same animals.

MATERIAL AND METHODS

Animals : Pathogen free female Sprague Dawley rats, strain OFA, average body weight 205g, were obtained from IFFA Credo (France) and divided into three equal groups (25 rats each). Group 1 received an intraperitoneal injection of 0.2ml CCl_4 in paraffin oil (total volume 1ml) Group 2 received 0.4ml CCl_4 in the same conditions while group 3 received paraffin oil alone and served as control. All the animals had free access to food (UAR pellets) and water.

Sacrifices and sampling: Four rats of each group were sacrificed at 5, 16, 24, 48, 72 and 96 hrs after carbon tetrachloride injection. The animals were killed under ether anesthaesia. Blood was collected by puncturing the abdominal aorta and was allowed to coagulate at room temperature for 2hrs. Following centrifugation (2500g x 15mn) serum samples were divided into aliquots and stored at -20°C until processed. In each series, animals were weighed, the liver was promptly removed weighed and immersed in Bouin's fixative.

Histology : Pieces of liver fixed in Bouin's solution were embedded in paraffin and cut at 4u following the usual procedure. Microscopic slides were stained using hematoxilin-eosin.

Hormone assays : Individual serum samples in duplicate were extracted with ether and assayed for estradiol and progesterone by a radioimmunoassay kit from the Commissariat à l'énergie atomique (CEA, Saclay, France) . Cortisol and thyroxin were assayed on serum samples using RIA kits from CEA and Corning (Medfield, USA) respectively.

AFP assay: AFP was assayed by the enzymo-immunological method previously described (6) using pure AFP as standard.

RESULTS

Fig:1 shows the effect of carbon tetrachloride intoxication on body weight, liver weight and AFP synthesis. In the control group, all animals maintained body weight. Following acute intoxication with CCl_4 all animals showed a loss in body weight during the first 24hrs then, a progressive return to normal values was observed. Liver weight was quite stable in control rats, while an increase was observed in both CCl_4 treated groups. AFP serum level (43ng/ml in the control group) was largely increased by CCl_4 treatment. The augmentation of the AFP serum level was observable on the 48th hr and persisted on the 96th hr.

Fig:2 shows the hormonal levels during CCl_4 exposure. Estradiol levels

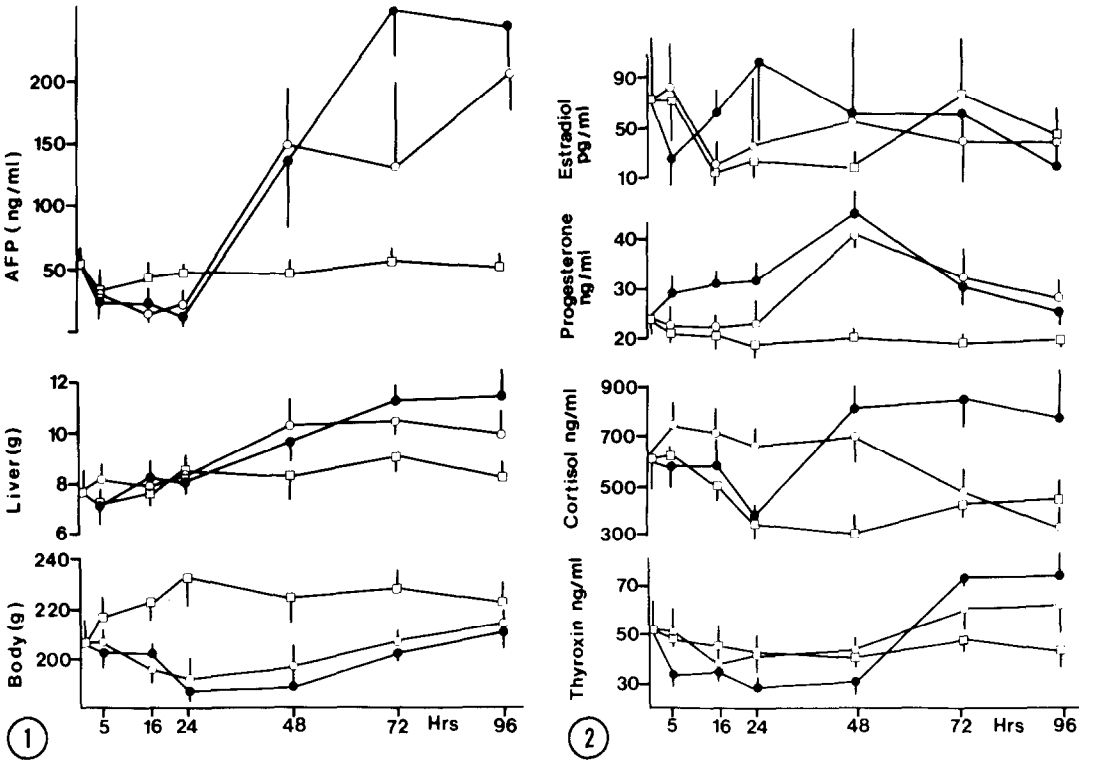

Fig. 1. Body weight, liver weight and serum alpha-fetoprotein levels in rats following CCl_4 induced liver injury.
Each point represent the mean of four rats and vertical bars correspond to standard deviation. □ control rats receiving 1ml paraffin oil. ○ rats treated with 0.2ml or ● 0.4ml CCl_4 .

Fig. 2. Serum hormone levels in rats following CCl_4 induced liver injury.
Groups of 25 rats were intraperitoneally injected with 0.2ml ○ or ● 0.4ml CCl_4 dissolved in liquid paraffin. Control rats □ received paraffin alone. Data is expressed as the mean of four rats ± standard deviation.

remained normal (10 to 90 pg/ml) in the three groups of rats. Progesterone levels presented a peak on the 48th hr in CCl_4 treated animals. This peak was about 2 times higher than the progesterone concentration in control rats. Nevertheless, variations of this hormone remained in the normal range for rats (10 to 50 ng/ml) (3,7). Cortisol levels were increased in the two groups of rats treated with CCl_4. The normal range of cortisol concentration for female Sprague Dawley rats (8) was 250 to 600 ng/ml. In the present work, rats treated with 0.2ml CCl_4 presented values above 750ng/ml at 6hrs then this level dropped to reach 300 ng/ml at 96hrs. Conversely, rats treated with 0.4ml

CCl_4 presented a normal level of cortisol during 24hrs then, this level was increased above 800 ng/ml on the 96th hr. Thyroxin levels remained normal (30 to 55 ng/ml) in the three groups of rats up to the 48th hr then, this level rose on the 72th and 96th hr in both CCl_4 treated groups. The histological study showed a massive necrosis of liver parenchyma from the 5th to the 96th hr. Regenerative foci and biliary canaliculi proliferation was observed both on the 72th and the 96th hr.

DISCUSSION

This study shows that whatever the dose of CCl_4 used, AFP is synthetised and secreted into the blood 48hrs after liver intoxication. The same result was previously obtained with the hepatocarcinogen N-2-fluorenylacetamide (3) and confirm that 48hrs is the latency time necessary to produce an elevation of AFP in adult rats. An other point is the relatively low level of AFP obtained after CCl_4 induced liver injury. This may be due to the use of adult rats instead of rats in development as generally used (9, 10). Moreover, it is known that adult rats synthetise less AFP than developping ones when treated with hepatotoxics (11, 12). Watanabe et al(9) described the AFP response as a function of the dose of CCl_4. In our hands, this dose response relationship was inexistant on the 24th hr after CCl_4 intoxication but appeared both on the 72th and the 96th hr confirming the result of these authors.

A second point is the absence of variation of the estradiol level despite the important elevation of the estrogen binding capacity by serum AFP. This was previously observed in rats treated with N-2-fluorenylacetamide and might be explained both by the action of AFP on the ovaries and the toxic action of CCl_4 on these glands (3).

Progesterone and thyroxin levels were increased in CCl_4 treated rats and the elevation observed were before and during liver regeneration respectively as demonstrated by the histological study. No evidence, for a decrease of AFP serum level when thyroxin is increased, was obtained. Thus, actually, we are not able to confirm a physiological regulation of AFP synthesis by thyroxin. The decrease of the AFP level observed by Belanger et al (1) might be due to a pharmacological effect due to the dose of thyroxin used (3ug/day) . Morley et al (5) have shown that thyroxin dropped after partial hepatectomy and subsequent liver regenera-

tion and concluded that this hormone was not stimulator of cell proliferation . In the present work, a different figure was found as thyroxin level was increased concomitantly with liver regeneration demonstrated by the histological study . This rises the problem of a different hormonal regulation depending on the type of liver attack before regeneration .

REFERENCES

1) Belanger, L., Hamel, D., Lachance, L., Dufour, D., Tremblay, M. and Gagnon, P. M.; (1975) Nature, 256, 657-659.
2) De Nechaud, B., Becker, J. E. and Potter Van, R. (1976) Biochim. Biophys. Res. Commun. 68, 8-15.
3) Aussel, C., Stora, C., Lafaurie, M., Krebs, B. and Ferrua, B. (1979) Steroids, 34, 631-642.
4) Pool, T. B., Hagino, N. and Cameron, I. L. (1978), J. Reprod. Fert. 53, 39-44.
5) Morley, C. G. D., Kuku, S., Rubinstein, A. H. and Boyer, J. L. (1975) Biochim. Biophys. Res. Commun. 67, 653-661.
6) Maiolini, R. and Masseyeff, R. (1975) J. Immunol. Methods, 8, 223-234.
7) Horikoshi, H. and Susuki, Y. (1974) Endocrinol. Japon. 21, 69-79.
8) Lafaurie, M., Aussel, C., Stora, C. and Krebs, B. (1980) Steroids , in press
9) Watanabe, A., Miyasaki, M. and Taketa, K. (1976) Gann, 67 , 279-287.
10) Watanabe, A, Miyasaki, M. and Taketa, K. (1976) Cancer Res. 36, 2171-2175.
11) Gilli, J. and Masseyeff, R. (1974) in l'Alpha-fetoproteine, R. Masseyeff Ed, INSERM, 231-239.
12) De Nechaud, B. and Uriel, J. (1971) Int. J. Cancer, 8, 71-80.