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SHORT COMMUNICATION

TESTOSTERONE SECRETION IS SEVERELY IMPAIRED AFTER HEPATECTOMY IN RAT, DOG AND MAN‡

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Summary—In 90%-hepatectomized rats, the plasma testosterone level (0.34 ± 0.07 ng.ml, mean ± SEM) is significantly lower ($P < 0.001$) than in sham operated male rats (1.7 ± 0.26 ng.ml, mean ± SEM). In dogs, after 90% hepatectomy, the mean plasma testosterone concentration fell to $\frac{1}{10}$ of the plasma testosterone level measured in sham operated animals either 24 or 72 h after surgery. In hepatectomized men, plasma testosterone is markedly decreased in contrast to what is observed after duodeno-pancreatectomy performed under the same conditions of anesthesia.

These results suggest that 90% hepatectomy severely alters the Leydig cell function.

INTRODUCTION

Complete liver regeneration is possible following single stage 90% hepatectomy in rats pretreated with testosterone-enanthate [1]. Eighty percent of the pretreated rats undergoing 90% hepatectomy were alive 40 h later and half of this population had a normal lifespan. In contrast, most of the rats which were not pretreated died before hour 40 following surgery. Some animals survived which provided an opportunity for studying the influence of hepatectomy on plasma testosterone concentration. The same study was also performed on 90% hepatectomized dogs and in men undergoing hepatectomy for liver cancer. The liver constitutes the principal site for androgen catabolism and inactivation. Hepatectomy should therefore drastically alter the metabolic clearance of testosterone and increase its concentration in the plasma. We thus tested the repercussions of 90% hepactectomy on Leydig cell function. Our results show that plasma testosterone is markedly decreased by single stage 90% hepatectomy in the rat, the dog and man.

EXPERIMENTAL

Adult male Wistar rats (average weight 200 g) were kept in a light-controlled environment (light from 07:00 to 19:00) and fed A 03 chow (UAR; 91360 Epinay-sur-Orge, France). The animals were divided into two groups. Group 1 (19 animals) underwent 90% hepatectomy: seven were sacrificed immediately (T), six at 24 h and six at 36 h after hepatectomy. Group 2 (12 animals) underwent a sham operation: six were sacrificed at 24 h and six at 36 h. Blood was collected in heparinized tubes and centrifuged at 4°C. The plasma was stored at −20°C for duplicate testosterone and corticosterone assays. Surgery was performed between 8 a.m. and 11 a.m. The animals received no solid food 12 h before and 36 h after surgery but were allowed 5% dextrose in water *ad libitum*. Anesthesia was produced by ethyl-ether oxygen inhalation. The surgical procedure is described in [1]. The animals regained consciousness 5 min after surgery and remained in metabolic cages for the duration of the study.

Plasma testosterone was measured by radioimmunoassay. Plasma samples (50–100 μl) were extracted with 3 ml of ethyl-ether using a magnetic stirring system for 3 min. Two ml of ether extract was evaporated to dryness under a nitrogen stream at room temperature. Testosterone was determined using reagents from CEA-Sorin (B.P. 21, 91190 Gif-sur-Yvette, France) following an unmodified protocol provided by the manufacturer. Antisera were obtained by immunization against a serum-albumin testosterone conjugate (linked at position 6 on the steroid ring). The free and bound fractions were separated by dextran-coated charcoal. This method is sensitive to 2.5 pg in the sample. For testosterone levels between 0.4 and 4 ng/ml, the "intra-assay" and the "inter-assay" coefficients of variation were 6 to 10% respectively.

Plasma corticosterone was determined by radio-competition using a slightly modified Murphy method [2]. The "intra-assay" and the "inter-assay" coefficients of variation were 8 and 12% respectively, and the sensitivity of the method was 2.5 μg/100 ml.

Adult male dogs (without pedigree) underwent 90% hepatectomy (7 animals) or a sham operation (5 animals): blood samples were collected and analyzed under the same conditions as for the rats.

In the men, blood samples were obtained during biological tests of 4 patient (57 ± 6 years old) undergoing hepatectomy for liver cancer. Biological follow up studies after duodeno-pancreatectomy in patients aged 53 ± 8 served as controls.

RESULTS

In the control rats and dogs, plasma testosterone was normal. In the men, plasma testosterone was in the normal range before surgery owing to the age of the subjects. In the

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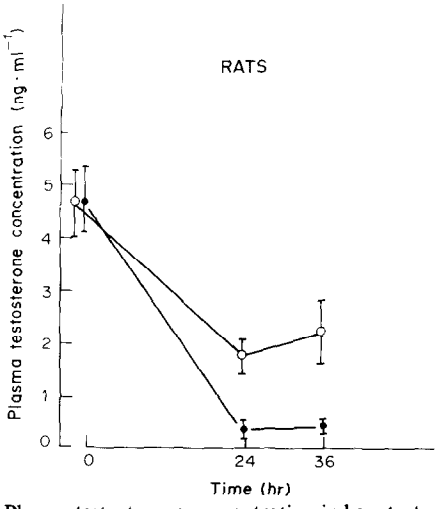

Fig. 1. Plasma testosterone concentration in hepatectomized (●) or sham operated (○) adult male rats. Animals were hepatectomized or sham operated at T0. Seven animals were sacrificed at T0, six at 24 and 36 h respectively in each group. Values are the mean ± SEM.

castrated rats, plasma testosterone ranged between 0.04 and 0.18 ng/ml (0.10 ± 0.04, mean ± SEM, $n = 6$).

Figures 1, 2, and 3 show that plasma testosterone concentration is markedly altered after hepatectomy in the rat, the dog, and man.

In the rats, (Fig. 1) at 24 h post hepatectomy, the plasma testosterone concentration was drastically reduced to 0.34 ± 0.07 ng.ml (mean ± SEM, $n = 6$), and remained at this level until hour 36, when it was 0.39 ± 0.06 ng.ml (mean ± SEM, $n = 6$). In sham operated rats, the plasma testosterone concentration decreased: 1.7 ± 0.26 ng.ml (mean ± SEM, $n = 6$), but remained at levels significantly higher ($P < 0.001$) than those in hepatectomized animals 24 h after surgery. The plasma corticosterone concentration did not vary greatly 0.37 ± 0.9, 0.39 ± 0.3 and 0.58 ± 0.12 $\mu g \times ml^{-1}$ at respectively 0, 24 and 36 h after hepatectomy. The small rise observed at 36 h is probably related to the nyctohemeral rise of corticosteroids corresponding to the end of the diurnal period of the animal.

The same observations hold for the dogs (Fig. 2). After 90% hepatectomy, the mean plasma testosterone concentration fell to $\frac{1}{20}$ of the mean value observed before surgery and remained at about $\frac{1}{10}$ of the plasma testosterone level measured in sham-operated animals either at 24 or 72 h.

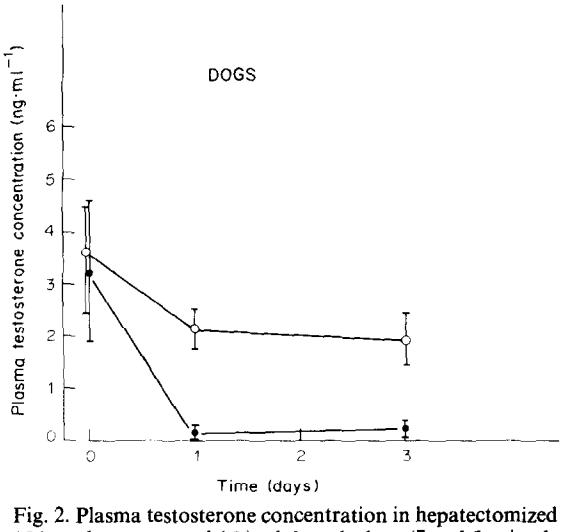

Fig. 2. Plasma testosterone concentration in hepatectomized (●) or sham operated (○) adult male dogs (7 and 5 animals respectively). Values are the mean ± SEM.

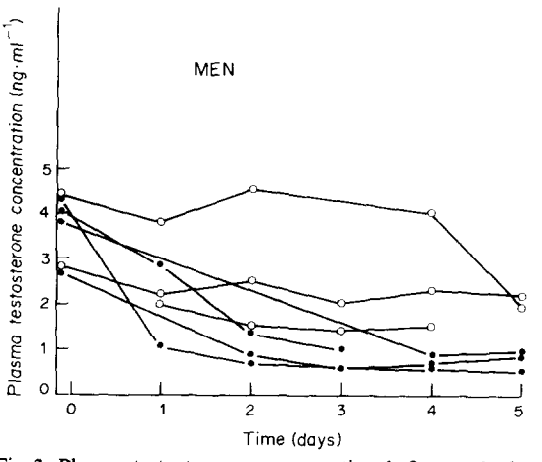

Fig 3. Plasma testosterone concentration before and after hepatectomy (●) or duodeno-pancreatectomy (○) in seven adult men.

In hepatectomized patients, plasma testosterone was markedly decreased, in contrast to what was observed after duodeno-pancreatectomy (Fig. 3)

In all the patients undergoing hepatectomy, plasma testosterone values remained at lower than 1 ng per ml for several days after hepatectomy.

DISCUSSION

The liver plays a major role in the metabolic clearance rate of androgens. A rise in plasma testosterone resulting from a decreased clearance rate was expected after 90% hepatectomy. However, plasma testosterone levels were drastically reduced after single stage hepatectomy in rats, dogs and men. This decrease could be related to a concomitant decrease in testosterone secretion, since activation of metabolic clearance cannot be evoked following hepatectomy. Plasma testosterone decreased in both hepatectomized and sham operated rats and dogs. This common effect can be attributed to a secondary effect of ether anesthesia which rapidly decreases plasma testosterone [3]. Ether anesthesia reduces testosterone secretion, probably by decreasing testicular blood flow [4]. However, ether anesthesia cannot be the only agent responsible for completely blocking testosterone secretion in hepatectomized animals for the following reasons: (a) In our studies, the plasma testosterone levels in the hepatectomized animals remained five times lower than those of the sham operated rats ($P < 0.001$). (b) The influence of residual ethyl ether is unlikely since the hepatectomized animals, as well as the sham operated rats and dogs, awoke within a few minutes after surgery and had apparently normal behavior afterwards. (c) In the dogs, the plasma testosterone level of the controls was less altered than in the rats, and in the men, plasma testosterone was not altered after duodeno-pancreatectomy performed under the same conditions of anesthesia. This strongly suggests that, besides ether anesthesia, 90% hepatectomy *per se* contributes to the cessation of testosterone secretion.

The stress and concomitant stimulation of the adrenocorticotropic axis could be put forward to explain the inhibition of testis function. However, this effect is unlikely since no rise in plasma corticosterone was detectable in the rats 24 h after hepatectomy and the rise at 36 h was within the range of the nyctohemeral variation in these animals. Furthermore, the plasma testosterone decrease persisted as long as 3 and 5 days in the dogs and the men respectively.

The persistence of corticoid secretion in the hepatectomized animals indicates that a general alteration of endocrine function by systemic alterations in blood flow or blood pressure is an unlikely explanation for drastic plasma testosterone decrease. Similarly, owing to the duration of the survival of the dogs and men, it is difficult to propose a general poisoning of the organism by toxic metabolites normally eliminated by the liver.

Retinoids [5], SHBG (sex hormone binding globulin) [6], lipoproteins [7] are dependent from liver function and implicated in testosterone metabolism; they could be involved in the damage of Leydig cell function observed after hepatectomy in animal and man.

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