Influence of chronic phenobarbitone treatment on uterine phosphofructokinase induction

SIR,—Administration of oestradiol-17β was shown to produce a rapid increase in phosphofructokinase activity in the uterus of the ovariectomized rat (Singhal & Valadares, 1966). A significant increase in enzyme activity was observed at 4 hr and peak levels were reached 16 hr after administration of a single intramuscular injection of oestradiol-17β (10 μg/100 g). Actinomycin, puromycin, ethionine, cycloheximide and 5-fluorouracil all blocked this hormone-induced response, suggesting that both new RNA and new protein syntheses are involved in the observed increases in uterine phosphofructokinase activity (Singhal & Ling, 1966b; Singhal & Valadares, 1967; Singhal, Valadares & Ling, 1967).

The ability of phenobarbitone to enhance drug metabolism by increasing the activity of liver microsomal enzymes is well documented (Remmer, 1962; Conney & Burns, 1962). Pretreatment of immature female rats with phenobarbitone for several days inhibits the uterotrophic response of tritiated oestradiol and decreases the concentration of the labelled oestrogen in the uterus (Conney, 1967; Levin, Welch & Conney, 1967). The stimulatory effect of chronic phenobarbitone administration on oestradiol metabolism was shown to result in an inhibition of the oestradiol-induced increase in uterine wet weight and in the incorporation of [^{14}C]glycine into uterine protein (Levin & others, 1967). We now report the effects of phenobarbitone treatment on oestrogen-induced increases in the activity of uterine phosphofructokinase.

Mature female Wistar rats, 180–200 g when killed, were ovariectomized bilaterally under light pentobarbitone anaesthesia. Two weeks later the following groups of ovariectomized rats were used: (1) control rats injected with saline solution; (2) animals injected with phenobarbitone; (3,4,5) rats administered oestradiol-17β in doses of 2·5, 5·0 or 10·0 μg/100 g respectively; (6,7,8) animals injected intraperitoneally with phenobarbitone (37·0 mg/kg) twice daily for 3 days before the administration of 2·5, 5·0 or 10·0 μg/100 g of

TABLE 1. THE EFFECT OF PHENOBARBITONE PRETREATMENT ON OESTROGEN-INDUCED ENZYME SYNTHESIS IN UTERI OF OVARIECTOMIZED RATS

Treatment	Uterine weight (mg)		Phosphofructokinase activity	
Control	110 ± 0 (100)		6·5 ± 0·1 (100)	
Phenobarbitone	126 ± 7 (115)		5·9 ± 0·1 (90)	
	Oestrogen-injected rats			
	Without phenobarbitone	With phenobarbitone	Without phenobarbitone	With phenobarbitone
Oestradiol-17β				
2·5 μg/100 g	220 ± 14 (200)*	158 ± 5 (143)*†	19·0 ± 0·5 (292)*	7·9 ± 0·3 (122)†
5·0 μg/100 g	219 ± 14 (199)*	160 ± 1 (146)*†	20·6 ± 1·2 (317)*	10·2 ± 0·4 (150)*†
10·0 μg/100 g	293 ± 47 (207)*	222 ± 17 (201)*	26·0 ± 0·5 (400)*	12·7 ± 0·9 (196)*†

Each value for PFK represents the mean ± s.e. based on 3 determinations of enzyme activity in uteri pooled from 2–3 rats. Rats were treated with 37 mg/kg of phenobarbitone intraperitoneally, twice daily for 3 days. Various doses of oestradiol-17β were administered intramuscularly to different groups of rats 16 hr before death. Enzyme activity is calculated as μmoles of alkali-labile phosphate formed per g of tissue per hr at 37°C × fresh weight of the tissue. Figures are in percentages taking the values of control rats as 100%.

* Statistically significant difference compared to the values of control rats ($P = <0{\cdot}05$).

† Statistically significant difference compared to the corresponding group of oestradiol-injected rats without phenobarbitone treatment ($P = <0{\cdot}05$).

oestradiol. All animals receiving oestradiol were injected 16 hr before death. Uteri were excised, cleaned of all adhering tissue and weighed rapidly on a Roller Smith torsion balance. The uteri were pooled, finely minced with scissors and 5% homogenates were then prepared in isotonic KCl solution of pH 7·4. The supernatant fluid was obtained and phosphofructokinase activity assayed under linear kinetic conditions as described previously (Lea & Walker, 1965; Weber & Singhal, 1965). Enzyme activity was calculated as μmoles of alkali-labile phosphate formed per hr per g of tissue at 37° times the weight of the organ (Singhal, 1967; Singhal & others, 1967). Statistical significance was calculated by Student's t test; a P value $<0{\cdot}05$ was considered significant.

Table 1 summarizes the effect of phenobarbitone administration on oestrogen-induced changes in uterine wet weight and phosphofructokinase activity. Treatment with phenobarbitone alone was without any significant effect on either of these two parameters, since values obtained after its administration were similar to those of saline-injected control rats. Uterine wet weights were increased to 200, 199 and 267% of the controls by 2·5, 5·0 and 10·0 μg/100 g of oestradiol respectively. In contrast, rats pretreated with phenobarbitone, but receiving the above doses of oestradiol, had increased uterine weights of only 143, 146 and 201%. Uterine phosphofructokinase activity was increased to 292, 317 and 400% of the control group in rats treated with 2·5, 5·0 and 10·0 μg/100 g of oestradiol-17β. Pretreatment with phenobarbitone blocked almost completely the oestradiol-induced enzyme increase by the 2·5 μg/100 g dose. However, the increases in phosphofructokinase activity induced by 5·0 and 10·0 μg/100 g of oestradiol were inhibited partially by phenobarbitone, and enzyme activity in these latter groups of animals increased to only 150 and 196% respectively.

Conney & others have shown that treatment of animals with phenobarbitone for several days increased the activity of liver microsomal enzymes which hydroxylate oestrogens (Kuntzman, Jacobson & others, 1964; Conney, Schneidman & others, 1965) and glucocorticoids (Conney & Schneidman, 1964; Conney, Jacobson & others, 1965). An earlier report from this laboratory has shown that pentobarbitone effectively prevented triamcinolone-induced increases in the activities of hepatic glucose 6-phosphatase and fructose 1,6-diphosphatase (Singhal & Ling, 1966). It is likely that this inhibition of glucocorticoid-induced liver gluconeogenic enzyme synthesis by pentobarbitone and the interference with oestrogen-induced biochemical responses by phenobarbitone described here may both be due to the stimulation of microsomal hydroxylases necessary for the inactivation of steroid hormones.

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Haemorrhagic, traumatic and tourniquet shock in the rat

SIR,—Gecse, Karady & West reported in 1964 that one colony of Wistar rats (termed non-reactors) was genetically more resistant to tourniquet and traumatic shock than were other colonies (termed reactors). We have now considered whether these differences can be accounted for by differences in the reactivity of their plasma kinin systems, especially as bradykinin is known to be involved in some forms of shock (Rocha e Silva & Antonio, 1960; Brocklehurst & Lahiri, 1962).

Groups of 10 non-reactor Wistar rats, weighing 150–200 g, obtained from the Agricultural Research Council's Field Station at Compton, and groups of 10 reactor Wistar rats from Fison's Ltd., Holmes Chapel, were subjected to haemorrhagic shock by the withdrawal of 15 ml blood/kg, traumatic shock (Noble & Collip, 1942), or tourniquet shock (Wilson & Roome, 1936). At different times after these procedures, the circulating levels of free kinin, kininogen, kininase and kinin-forming enzymes were measured (Dawson, Starr & West, 1966). Plasma kininogen level was the only parameter to show consistent changes and these occurred within 10 min of each type of shock; for example, the levels of the kinin precursor in reactor rats increased about three-fold but these were not sustained and returned to control values by 30–60 min. These changes in kininogen are similar to those reported by Diniz & Carvalho (1963) during haemorrhagic shock in the dog. Non-reactor rats showed similar changes in haemorrhagic and traumatic shock (25 min at 40 rev/min) but not in tourniquet shock (4 hr duration) where the kininogen levels were not raised during the experimental period. The plasma kinin systems in liver, heart, lung and small intestine were also unchanged after each type of shock in both types of rat.

Rats dying after severe shock always showed intestinal haemorrhage and experiments were therefore made to study kinin release into the peritoneal cavity, where it may arise from activation of its precursor by the action of kinin-releasing enzymes originating from pro-enzymes in the stagnating blood or from stores in the walls of the intestine. Immediately after subjecting other groups of rats to the different shock procedures, therefore, the peritoneal cavity of each rat was washed with 5 ml of 0·9% (w/v) saline and the washings were assayed for kinin-like activity. Whereas at all times after haemorrhagic shock, the bradykinin levels in the peritoneal fluid did not increase above the basal values (about 10 ng), the levels after the other two types of shock increased, the extent depending upon the intensity of the shock applied (Table 1).