Effect of pentobarbitone administration on corticosteroid-induced synthesis of hepatic gluconeogenic enzymes

SIR,—Administration of glucocorticoids in rats caused preferential increases in liver gluconeogenic enzymes without affecting other enzymes involved in carbohydrate metabolism (Weber & Singhal, 1964a; 1964b). That the steroid-induced increases observed for hepatic glucose 6-phosphatase and fructose 1,6-diphosphatase were due to new enzyme synthesis was revealed in recent investigations in which actinomycin, puromycin and ethionine abolished these increases (Weber & Singhal, 1964b; Weber, Singhal & Srivastava, 1965a). More recently, evidence has been obtained that glucocorticoid hormones function as inducers and insulin as a suppressor of biosynthesis of key, hepatic gluconeogenic enzymes (Weber, Singhal & Srivastava, 1965b; Weber, Singhal, Stamm & Srivastava, 1965; Singhal & Weber, 1965).

In the course of recent experiments designed to demonstrate the *direct* effect of steroids (injected via the portal vein) on the activities of these gluconeogenic enzymes in the livers of dogs anaesthetised with pentobarbitone, we found there was no change in these enzyme activities over a 6 hr period. This observation was surprising in view of previous data demonstrating rapid increases in both enzymes after intraperitoneal administration of glucocorticoid hormones in rats (Weber & Singhal, 1964a; Weber & Singhal, 1964b). From these observations, it appeared possible that barbiturate anaesthesia may have interfered with glucocorticoid-induced enzyme synthesis. To test this possibility, experiments were designed in which triamcinolone-induced enzyme synthesis was studied in rats in the absence and presence of pentobarbitone. The results obtained show that pentobarbitone treatment of rats injected with triamcinolone largely prevented the increases in hepatic glucose 6-phosphatase and fructose 1,6-diphosphatase activities.

Male Wistar rats weighing 100–120 g and maintained on Master Laboratory Chow and water *ad libitum* were divided into the following 4 groups: (1) control rats injected with saline; (2) animals injected with pentobarbitone; (3) and (4) triamcinolone-treated rats without and with pentobarbitone administration. Triamcinolone (1 mg/100 g) was injected intraperitoneally every 24 hr for 3 days. Pentobarbitone (1·5 mg/100 g) was also injected intraperitoneally at 12 hr intervals during the 3 day period. In addition, a final injection of pentobarbitone (1·5 mg/100 g) was given 2 hr before the rats were killed. Rats in all groups were killed on the morning of the 4th day. Activities of liver glucose 6-phosphatase and fructose 1,6-diphosphatase were assayed in homogenates and supernatant fluids respectively, according to procedures previously described (Weber, Singhal & Stamm, 1963; Weber & Singhal, 1964b). Protein was estimated using the method of Lowry, Rosebrough, Farr & Randall (1951) and blood sugar determinations were made by the method referred to by Weber & Singhal (1964c) Enzyme activities are expressed as total available activity (Weber & Singhal, 1964a, b) and calculated as μmoles of substrate metabolised per g of liver × liver to body weight ratio × 100. The data were subjected to statistical evaluation as described previously (Weber & Singhal, 1964a; Singhal & Valadares, 1966).

Table 1 summarises the effect of pentobarbitone administration on hepatic glucose 6-phosphatase, fructose 1,6-diphosphatase, total protein and blood sugar level in rats treated with triamcinolone for 3 days. Treatment with pentobarbitone alone was without effect on the parameters studied, since values obtained after its administration were similar to those of saline-injected controls. In triamcinolone treated rats, hepatic glucose 6-phosphatase was increased to 205% and fructose 1,6-diphosphatase to 199% of controls. However, in rats treated concurrently with triamcinolone and pentobarbitone, hepatic glucose 6-phosphatase and fructose 1,6-diphosphatase increased to only 119% and 136%

TABLE 1. EFFECT OF INTRAPERITONEAL PENTOBARBITONE ON TRIAMCINOLONE-INDUCED SYNTHESIS OF HEPATIC GLUCONEOGENIC ENZYMES IN WISTAR RATS

Parameter examined	Control	Pentobarbitone	Triamcinolone-treated rats	
			Without pentobarbitone	With pentobarbitone
Glucose 6-phosphatase ..	5187 ± 86 (100)	4928 ± 49 95	10,639 ± 638 205* (100)	6190 ± 536 119; 58†
Fructose 1,6-diphosphatase	2245 ± 49 (100)	2335 ± 104 104	4452 ± 292 199* (100)	3049* ± 300 136; 69†
Total protein	303 ± 18 (100)	342 ± 31 113	440 ± 15 145* (100)	401 ± 14 132*; 91
Blood sugar (mg/100 ml) ..	107 ± 3 (100)	136 ± 10 127	145 ± 11 136* (100)	132 ± 6 123*; 91

Means ± s.e. represent 4 or more animals in each group.   Enzyme activities are calculated as μmoles of substrate metabolised per g of liver × liver to body weight ratio × 100.   Total protein was estimated in the supernatant fluid and expressed as mg of protein per g of liver × liver to body weight ratio × 100.   Data are also given in percentages taking the values of control rats as well as for triamcinol one-injected animals without pentobarbitone administration as 100% (in parentheses).
  * Statistically significant difference as compared to the values of control rats (P = < 0·05).
  † Statistically significant difference as compared to the values of triamcinolone-treated rats without pentobarbitone administration (P = < 0·05).

respectively. The increases observed in total protein content and blood sugar leve after triamcinolone treatment were not significantly affected by pentobarbitone.

The possibility that mild generalised sedation produced by pentobarbitone (1·5 mg/100 g every 12 hr) may have been responsible for the observed effects cannot be ruled out; at present we are not certain whether the observed interference in triamcinolone-induced enzyme synthesis can be achieved by other agents with known central depressant effects.   It should also be emphasised that although the animals treated with pentobarbitone displayed some decrease in motor activity, their food intake appeared to be normal.   It is likely that the observed inhibition of glucocorticoid-induced enzyme synthesis by pentobarbitone may have been due either to a stimulation of a system necessary for inactivation of the steroid hormone or the effects of a direct interaction between triamcinolone and the barbiturate.

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