PARADOXICAL REFRACTORINESS TO INDUCTION BY CORTISONE FOLLOWING A PECULIAR ADAPTATION OF LIVER ENZYMES BY BENTONITE OR CELITE.

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SUMMARY

Repeated administration of bentonite or celite results in sustained induction of liver tryptophan pyrrolase and tyrosine transaminase activities. Such peculiarly pronounced and precocious adaptation of selected liver proteins is not a consequence of: a) any unusual or marked toxicity, or particularly obnoxious nature, of the conditioning substance, b) association with generally enhanced liver synthetic activity and c), most importantly, enzyme proteins thus affected are subsequently refractory to the otherwise inductive influence of a glucocorticoid hormone.

Studies with stress inducing agents and/or metabolic variants of various types have been very helpful in revealing aspects of enzyme regulation in mammalian liver (1-5). Of possible fundamental significance, among these, has been the use of materials capable of altering Kupffer cell function since this has lead to the concept that the reticuloendothelial system (RES) of an animal seems to contribute, either directly or indirectly, to the maintenance of homeostasis in liver parenchymal cell (brief review in 6). Generally, an animal experiencing deranged enzymatic function can be "conditioned" to the adverse effects of a stress provoking agent. This tolerant animal is, then, refractory to a subsequent challenge with the stressor such that enzymatic derange-

ments do not ensue (7,8). In all these cases, however, the enzymatic regulation by a corticoid hormone remains unimpaired and enzymatic induction ensues in the conditioned, as in the normal, animal. The purpose of this report is to describe an unusual type of enzymatic adaptation whereby the enzymes, otherwise inducible, become entirely refractory to the inducive effect of the hormone.

Swiss, male mice (25 ± 2 gms) were injected intraperitoneally with 5 mg celite (Johns-Manville) or 5 mg bentonite (Microbiological Associates) every day for 5 days; others were given 0.5 mg cortisone acetate (Roussel), subcutaneously, in an identical schedule. Controls were given only saline and all injections were administered in a volume of 0.5 ml. Forty eight hours after the last injection in the series, animals from each group were given either saline or 5 mg cortisone acetate and the livers assayed for enzyme activities 6 hours thereafter. Food and water were available ad libitum during all this time but food was withdrawn 16-18 hours prior to enzyme assay. Organ weights were determined on a Sartorius automatic balance.

Procedures for determination of tryptophan pyrrolase (TP) and tyrosine transaminase (TT) activities have been previously described (6-8). The enzyme activities are expressed as μ moles of kynurenine/gm liver/hr for TP and μg p-hydroxyphenylpyruvic acid/mg liver/10 min for TT.

Data in Figure 1 show that liver TP activity was present at fully induced levels in mice given either bentonite or celite. Furthermore, whereas cortisone induced the enzyme in control animals, the hormone was entirely ineffective in inducing TP in mice treated with either of the two diatomaceous preparations. The response of another inducible liver

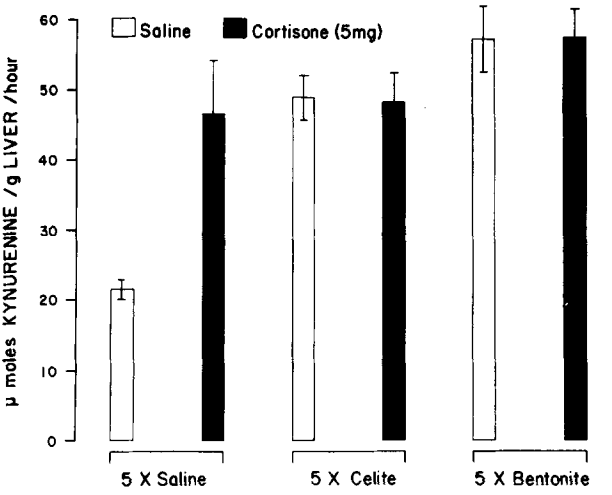

Figure 1. Refractoriness of liver tryptophan pyrrolase to the inductive effect of cortisone in mice pretreated with bentonite or celite. Each value is the mean, ± the standard error, of 8-11 individual determinations from replicate experiments. For details see text.

enzyme, under these conditions, is shown in Table I. It is evident that the behaviour of liver TT was no different than TP; the activity of TT was present at near induced levels in

Table I. Refractoriness of liver tyrosine transaminase to the inducive effect of cortisone in bentonite pretreated mice.

Treatment	Tyrosine Transaminase Activity§ (μg PHPP/mg Liver/10 min.)
CONTROL	4.43 ± 0.055
CORTISONE	8.58 ± 0.036
5 x BENTONITE	7.43 ± 1.000
5 x BENTONITE + CORTISONE	9.26 ± 0.055

§Each value is the mean, ± the standard error, of 5-6 individual determinations.

bentonite treated mice and this was not further influenced by cortisone.

The most obvious explanation for these results would be an adrenal - hypophyseal response, as a result of stress by celite or bentonite, resulting in increased glucocorticoid secretion which, in turn, would effect a continual synthesis of the enzymes. This was not substantiated in mice given 5, daily, subcutaneous injections of 0.5 mg cortisone. As shown in Table II, the activity of TP was normal 48 hours after the last injection (in the series of 5) with the hormone and cortisone was as effective in inducing TP in these mice as in normal animals. The induction levels recorded here are not the maximum attainable in the animal. Administration of cortisone with tryptophan results in much greater TP levels than were obtained with either inducer used in the present study.

Although this regimen of pretreatment with the hormone is

Table II. Lack of induced stabilisation of liver tryptophan pyrrolase by pretreatment with cortisone.

Treatment	Tryptophan Pyrrolase Activity[§] (μ moles Kynurenine/gm Liver/Hr)
5 x SALINE	21.7 ± 1.3 (26)
5 x SALINE + CORTISONE	46.8 ± 7.6 (6)
5 x CORTISONE	17.5 ± 2.2 (12)
5 x CORTISONE + CORTISONE	58.1 ± 5.2 (8)

[§]Each value is the mean, ± the standard error, of the number of individual determinations shown in parantheses.

more than adequate representation of hypercortical secretion that may ensue in mice treated with either bentonite or celite, it is realised, of course, that other aspects of pituitary - adrenal function associated with stress would probably escape analysis by this method.

Attention was next directed to the overall growth rate of these animals as compared to their normal counterparts. Data in Table III show that bentonite treated mice gained weight like the controls so the spleen or liver/body weight ratios were unchanged in such animals. Hence, the peculiar 'stabilisation' of particular enzyme proteins was not a reflection of an overall enhancement in the rate of liver synthetic activity. Contrarily, pretreatment with cortisone lead to pronounced spleen involution without any alteration in liver/body weight ratio and the hepatic enzyme adaptations did not ensue.

The results described here are uniquely different from those obtained with other materials which, like celite and bentonite, influence the RES. The differences between the

Table III. Unaltered spleen or liver/body weight ratios in bentonite pretreated mice.

	Body Wt	Liver Wt	Spleen Wt
CONTROL	25.0 ± 0.07	1.33 ± 0.08	0.15 ± 0.001
5 x BENTONITE	24.9 ± 0.63	1.29 ± 0.07	0.19 ± 0.003
5 x CORTISONE	25.2 ± 0.78	1.39 ± 0.08	0.08 ± 0.002

All values are the average, ± the standard error, of 8-10 individual determinations.

effects of various RE-active agents were first noted in experiments with single injections of any one of these substances. Whereas single injections of zymosan, glucan, endotoxin, all lower TP, initially, under similar conditions bentonite and celite do not influence TP at all (9,10). Upon repeated injections with zymosan, glucan, endotoxin, liver TP is stabilised - that is to say remains at the normal level upon challenge with the conditioning substance (9); cortisone can induce TP just as well in the conditioned, as in the normal animal (11,12). This is entirely different from the behaviour of TP as shown in Figure 1 where, upon repeated injections with either celite or bentonite, the enzyme is maintained at the induced level and is not further affected by cortisone. Neither can this behaviour be attributed to relative toxicities since 90-95% of the animals survived the 25 mg (total) dose of either bentonite or celite which is less toxic than an LD_{50} (750 µg) of endotoxin and the latter does not result in the peculiar enzymatic adaptation seen in the colloid pretreated mice (8,9). Since all these are particulate substances of comparable size, the nature of the material, too, does not seem to be responsible for the observed behaviour of TP. Hepatosplenomegaly, moreover, follows administration of endotoxin, zymosan, glucan (11), but this is not true of the agents used in the present study (Table III). Since some adrenal hypertrophy occurs after treatment with all agents except cortisone, it further shows that a mere enhancement in the endogenous secretion of glucocorticoids over a protracted period of time, alone, can not suffice as an explanation for the observed effects.

The possibility must be entertained that celite or bentonite may prolong the availability of metabolically active form of the

corticoids for more than the usual half life time, or alter the endogenous levels of substrates, cofactors, activators, or inhibitors which normally regulate TT and TP activities in vivo. If responsible, however, the hypophyseal - adrenal axis seems to be involved in a manner entirely different from those hitherto observed. In fact, as previously postulated, adrenal hormones, at best, seem to play a permissive role in the stress syndrome (13,14). It remains to be determined how a direct stimulation of the hypothalamus, or other aspects of pituitary - adrenal functions, may influence the course of stress that lead to the type of effects observed in the present study. Due to increased sensitivity, the response of hypophysectomised or adrenalectomised mice to treatments with bentonite or celite could not be assessed. The use of protein or RNA synthesis inhibitors may help delineate the various phases of enzyme induction that lead to the type of effects described here.

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