altered relationship between enzyme units and activity[12] as the induction of enzyme units appears to be normal[13]. The data suggests that thyroid hormone metabolism may be altered in *ob/ob* mice but be normal in GTG mice. Thus, it seems unlikely that the common defect in hepatic protein turnover in these 2 obese species is related to thyroid hormones, and the underlying mechanisms await further investigation.

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Inability of dithiols to cause activation of *Limulus* endotoxin sensitive procoagulase

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Summary. Using a chromogenic substrate it has been shown that the endotoxin sensitive procoagulase of *Limulus* lysate is not activated by dithiols. Increased turbidimetric readings in the presence of dithiols would therefore appear to be nonspecific.

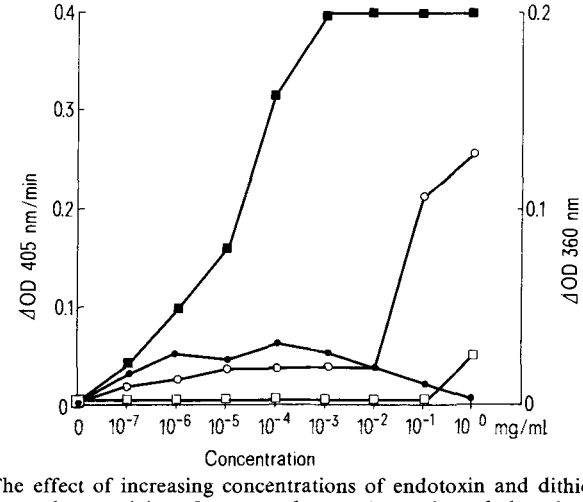
The *Limulus* amoebocyte lysate (LAL) method is currently used for the measurement of endotoxin in serum, biological and pharmacological products[1]. The use of the assay has come under some criticism since certain proteolytic enzymes, synthetic polynucleotides and peptidoglycans were found to give presumably false positive results[2-4]. Recently low molecular weight dithiols were reported to simulate endotoxin in the LAL assay at concentrations of 1 µg/ml or greater[5]. In this report we show that this apparent activity is not due to a specific activation of the *Limulus* lysate, as occurs in the presence of endotoxin.

Materials and methods. LAL was obtained from Sigma Chemical Co. and reconstituted in pyrogen free water. The LAL test was carried out according to a variation[6] of the chromogenic substrate method of Iwanaga et al.[7]. To 200 µl of 0.09 M Tris-HCl pH 8.0, 0.035 M $MgCl_2$ was added 150 µl of endotoxin or the dithiol, dithiothreitol (DTT) (Sigma) dissolved in saline, and 20 µl lysate. After 20 min at 37 °C in the LKB 2086 Mk II Reaction Rate Analyzer 50 µl of 1.25 mM chromogenic peptide substrate S2222 (Bz-Ile-Glu(γ-OR)-Gly-Arg-p-nitroanilide-HCl Kabi Vitrum Ltd) was injected and the change in optical density calculated using an LKB 2082 Kinetic Data Processor with fixed time programme. Similar mixtures were also made and incubated but without the addition of S2222, a turbidimetric measurement being made at 360 nm according to Platica et al.[5].

Results. The results (fig.) show a significant increase in turbidity at concentrations of dithiol greater than 10 µg/ml confirming the work of Platica et al.[5]. Much lower turbidity levels were observed by these workers in the presence of endotoxin and in fact in our system the dilution of lysate is such as to cause no increase in turbidity except at the highest concentration of endotoxin.

When the measurements are made using chromogenic substrate, however, endotoxin is shown to cause a rapid generation of enzyme reaching a peak at concentrations greater than 100 ng/ml. Increasing concentrations of DTT cause a much lower generation of enzyme reaching a peak approximately 10 times less than that observed with endotoxin. At concentrations of DTT at which a large increase in turbidity is observed there is a marked inhibition of enzyme activity which is total at 1 mg/ml.

Discussion. The highly sensitive endotoxin assay using amoebocyte lysate from the haemolymph of the *Limulus* crab rests on the observation that the solution gelates in the presence of endotoxin and using suitable conditions the rate of formation of a gel is dependent on endotoxin



The effect of increasing concentrations of endotoxin and dithiols upon the reactivity of *Limulus* lysate. Squared symbols refer to observations made in the presence of endotoxin, circular symbols to those made in the presence to DTT. Closed symbols refer to the change in absorbance at 405 nm and open symbols to the turbidimetric readings at 360 nm.

concentration[8]. The rate of gelation may also be measured turbidimetrically[9]. The mechanism involved in this reaction depends on the activation of a proenzyme by endotoxin[10]. The enzyme catalyzes cleavage of internal peptide bonds of a coagulogen converting it to an insoluble form[11]. Recently a chromogenic substrate has been shown to be split by the endotoxin activated enzyme in the lysates and this forms the basis of a new methodology[7].

In this report we have used this facility for detecting the endotoxin sensitive enzyme to show that dithiols do not activate the proenzyme form but instead cause a nonspecific precipitation of lysate protein which when observed turbidimetrically gives a false positive result. Measuring the enzyme activity whith chromogenic substrate much less activity is observed in the presence of dithiols than in the presence of endotoxins and in fact at the highest concentrations of dithiol, at which the turbidity increase is greatest, there is a reduction of enzyme activity.

A number of reports show that various reagents beside endotoxin may interfere in the LAL test. Of the proteolytic enzymes, trypsin has been shown to cause gelation of the coagulogen[2,11]. Thrombin was shown to interact[3] though this has since been partially refuted[12]. Various polynucleotides[3] and peptidoglycans[4] from Gram positive organisms have been shown to interfere though at quite high concentrations. Clearly such observations cast doubt on the use of the LAL test for endotoxin measurement. Using the chromogenic substrate method we have shown that dithiols, clearly shown to cause turbidimetric precipitation of the lysate[5], do so in a nonspecific fashion which does not involve activation of the endotoxin sensitive procoagulase.

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Prevention of estrogenic inhibition of adrenal Δ^5-3β-hydroxysteroid dehydrogenase by α_{2u}-globulin in rats

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Summary. The urinary protein α_{2u}-globulin stimulates adrenal Δ^5-3β-hydroxy-steroid dehydrogenase activity and prevents adrenal enlargement in estrogen-treated adult male rats.

Roy and Neuhaus[3] have isolated an androgen-dependent protein from male rats and identified it as α_{2u}-globulin. This protein is synthesized by the hepatic parenchymal cells in the adult male rats[4]. α_{2u}-globulin is absent in normal female rats[5] but can be induced in adult female rats by treatment with testosterone[6]. Estrogen treatment inhibits the synthesis of α_{2u}-globulin in mature male rats[7]. Estrogen also produces adrenal hyperplasia and results in an inhibition of Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSD) concerned with steroid hormone synthesis, in the adrenal of rats[8-10]. Since α_{2u}-globulin stimulates spermatogenesis[11] and testicular steroid hormone synthesis[12] in estrogen-treated male rats the present experiment has been undertaken to explore the effects of α_{2u}-globulin on adrenal Δ^5-3β-HSD in rats treated with estrogen.

Materials and methods. Adult laboratory bred male albino rats of the Sprague-Dawley strain weighing 150–250 g were used in this experiment. They were maintained at 30 °C with 10 h illumination daily, and given free access to Hindlever rat chow and water. α_{2u}-globulin was isolated from male rat urine following the procedure previously described[13]. 24 rats were divided into 3 groups. 2 groups of rats were treated with oestradiol-17β s.c. at a dose of 50 µg/100 g b.wt/day in propylene glycol for 7 days. At the end of treatment 1 group of estrogen-treated rats received s.c. injections of 1.5 mg of α_{2u}-globulin per day for 14 days while the other group of treated rats together with the control group were injected with vehicle only. Animals of all the 3 groups were sacrificed 24 h after the last injection. For studying the activity of Δ^5-3β-hydroxysteroid dehydro-

Role of α_{2u}-globulin on adrenal Δ^5-3β-HSD in estrogen-treated rats

Treatment	Initial b.wt (g)	Final b.wt (g)	Adrenal wt (mg/100 g b.wt)	Δ^5-3β-HSD activity (nmoles/mg/h)
Control	191.4 ± 23.5	219.6 ± 16.4	18.8 ± 0.9*	21.08 ± 1.21
Estrogen	176.8 ± 18.8	177.1 ± 16.2	25 ± 1.2	11.39 ± 1.16**
Estrogen + α_{2u}-globulin	180.8 ± 7.16	211.4 ± 7.5	20.3 ± 0.7	17.11 ± 0.99

Each value represents mean ± SE. * $p = 0.01$; ** $p = 0.001$ (Student's t-test) compared to control vs estrogen. All values control vs estrogen + α_{2u}-globulin statistically nonsignificant.