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.

genase, the adrenals were removed immediately and dropped into ice cold homogenizing medium consisting of equal parts of 0.9% saline and 0.1 M phosphate buffer, pH 7.4, to give a tissue concentration of 5 mg/ml. The enzyme was assayed by spectrophotometric measurement of the production of Δ^4 androstenedione from dehydroepiandrosterone (DHEA)[14].

Results. The table shows that the estrogen-treated rats grew at a slower rate than the controls. Estrogen injections caused a significant suppression of adrenal Δ^5-3β-HSD activity but produced enhanced adrenal weights as compared to controls.

When estrogen-treated rats received α_{2u}-globulin for 14 days, the rate of body growth was apparently normal. The weight of the adrenal gland was significantly decreased when estrogen-treated rats were given α_{2u}-globulin. The activity of Δ^5-3β-HSD was also increased to control levels in α_{2u}-globulin-treated animals.

Discussion. The experiments presented in this paper demonstrate that α_{2u}-globulin prevents adrenal enlargement and the inhibition of Δ^5-3β-HSD in estrogen-treated rats. A similar finding of adrenal hyperplasia followed by a decrease in Δ^5-3β-HSD activity has been reported in pregnant rats after estrogen injections[8]. Sanyal et al.[10] have suggested that the fall of Δ^5-3β-HSD activity in the inner 2 zones of the adrenal cortex may indicate suppression of corticosterone and androgen synthesis in estrogen-treated rats. Estrogen treatment[7] or adrenalectomy[15] significantly decreased α_{2u}-globulin synthesis in the adult male rats. Administration of this male urinary protein in estrogen-induced rats prevents spermatogenic degeneration[11] and stimulates testicular androgen synthesis[12]. The physiological role of α_{2u}-globulin in adrenocortical function has not been elucidated. The protection of Δ^5-3β-HSD activity and adrenal weight in estrogen-induced rat after α_{2u}-administration indicates that α_{2u} possibly by stimulating corticosterone synthesis prevents the excess release of adrenocorticotropic hormone which appears to cause adrenal hyperplasia in estrogen-treated rats[8].

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Irreversible inactivation of yeast glucose-6-P dehydrogenase by penicillin G[1]

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Summary. Yeast glucose-6-P dehydrogenase is irreversibly inactivated by penicillin G. Kinetic data show that 1 molecule of penicillin G reacts with each active unit when the enzyme is inactivated. The rate of inactivation increases greatly with increasing pH. This irreversible inactivation by penicillin G is largely prevented by pyridoxal-P, a reversible inactivator of this enzyme. Prior treatment of penicillin G with penicillinase totally abolishes its ability to inactivate the enzyme.

The basic structure of penicillins, as shown in figure 1, consists of thiazoline fused with a beta-lactam ring, to which variable side chains can be attached. The integrity of the beta-lactam ring is essential for anti-microbial activity of penicillins since its opening by penicillinase completely eliminates the effect[2]. It was proposed that the antimicrobial action of penicillin was due to the irreversible inactivation of transpeptidase by acylation of the enzyme active site[3]. Penicillins are well-known to induce hypersensitivity in some patients. The basis for this hypersensitivity is still not completely understood. A generally recognized hypothesis is that in solution penicillin may undergo molecular rearrangement to form a reactive intermediate, penicillenic acid, which may acylate ϵ-amino groups of lysyl residues of some proteins, thereby altering the protein antigenic properties (fig. 1)[4]. We have recently observed that yeast glucose-6-P dehydrogenase is extremely sensitive to inactivation by pyridoxal-P[5]. Since pyridoxal-P is highly specific for modifying the ϵ-amino groups of lysyl residues in proteins[6], it is considered that yeast glucose-6-P dehydrogenase contains lysine at its active site. This postulation prompted us to investigate whether this enzyme could be inactivated by penicillin G.

Materials and methods. Crystalline glucose-6-P dehydrogenase (from Bakers yeast), penicillinase (from *Bacillus cereus*), penicillin G (Na salt), and other chemicals used in this study were all purchased from Sigma Chemical Co., Saint Louis, MO, USA. The activity of glucose-6-P dehydrogenase was assayed as previously described[5]. Treatment of the enzyme (12 µg/ml) with different concentrations of penicillin G was carried out at 25 °C in 50 mM Na borate buffer at the pH values shown in the figure and table legends. Aliquots were removed at the times specified and immediately assayed for enzyme activity. Treatment of