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 $\Delta^4$ 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 $\Delta^5$-3$\beta$-HSD activity but produced enhanced adrenal weights as compared to controls.

When estrogen-treated rats received $\alpha_{2u}$-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 $\alpha_{2u}$-globulin. The activity of $\Delta^5$-3$\beta$-HSD was also increased to control levels in $\alpha_{2u}$-globulin-treated animals.

*Discussion.* The experiments presented in this paper demonstrate that $\alpha_{2u}$-globulin prevents adrenal enlargement and the inhibition of $\Delta^5$-3$\beta$-HSD in estrogen-treated rats. A similar finding of adrenal hyperplasia followed by a decrease in $\Delta^5$-3$\beta$-HSD activity has been reported in pregnant rats after estrogen injections[8]. Sanyal et al.[10] have suggested that the fall of $\Delta^5$-3$\beta$-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 $\alpha_{2u}$-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 $\alpha_{2u}$-globulin in adrenocortical function has not been elucidated. The protection of $\Delta^5$-3$\beta$-HSD activity and adrenal weight in estrogen-induced rat after $\alpha_{2u}$-administration indicates that $\alpha_{2u}$ 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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3 A.K. Roy and O.W. Neuhaus, Biochim. biophys. Acta *127*, 72 (1966).
4 A.K. Roy and D.L. Raber, J. Histochem. Cytochem. *20*, 89 (1972).
5 A.K. Roy and O.W. Neuhaus, Nature *214*, 618 (1967).
6 A.E. Sippel, P. Feigelson and A.K. Roy, Biochemistry *14*, 825 (1975).
7 A.K. Roy, D.M. Mcminn and N.M. Biswas, Endocrinology *97*, 1501 (1975).
8 M. Vogt, J. Physiol., Lond. *130*, 601 (1955).
9 A.S. Goldman, J. clin. Endocr. *28*, 231 (1968).
10 S. Sanyal, P.B. Patra and N.M. Biswas, Anat. Anz. *144*, 214 (1978).
11 A.K. Roy, J.G. Byrd, N.M. Biswas and A.K. Chowdhury, Nature *260*, 719 (1976).
12 N.M. Biswas, P.K. Ghosh and O.W. Neuhaus, unpublished observation.
13 A.K. Roy, O.W. Neuhaus and C.R. Harmison, Biochim. biophys. Acta *127*, 72 (1966).
14 B.L. Rubin, G. Leipsner and H.W. Deane, Endocrinology *69*, 619 (1961).
15 J.F. Irwin, S.E. Lane and O.W. Neuhaus, Biochim. biophys. Acta *252*, 328 (1971).

## 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 $\epsilon$-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 $\epsilon$-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

penicillin G (0.4 mmoles) with penicillinase (40 units) was carried out in 10 ml of 50 mM Na borate buffer for 15 min, and penicillinase was then removed by a Amicon ultrafilter (XM-50). During the reaction, the pH was maintained between 6.7 and 7.5 with 1 N NaOH.

*Results and discussion.* Yeast glucose-6-P dehydrogenase was incubated with various concentrations of penicillin G, and aliquots were removed at intervals and assayed for residual activity (fig. 2a). The inactivation reaction followed pseudo-first order kinetics with respect to enzyme. As originally introduced by Levy et al.[7] and subsequently used by many others[8–11], the number of inhibitor molecules reacting per active unit of enzyme to produce an inactive enzyme-inhibitor complex can be calculated by plotting the $\log(t_{0.5})^{-1}$ versus the log of penicillin G concentration and determining the slope of the line. When the data from figure 2a were plotted in this manner, a slope of 1.06 was obtained (fig. 2b). This suggests that the reaction of one molecule of penicillin G per active unit of the enzyme is necessary for inactivation.

The inactivation by penicillin G increased markedly with increasing pH. As shown in figure 3, incubation of the enzyme with 50 mM penicillin G at pH 8.0 for 30 min decreased the enzyme activity by more than 80%. This was compared with less than 10% decrease in activity when incubation was carried out at pH 6.8. It was found that prior treatment of penicillin G with penicillinase completely eliminated its ability to inactivate the enzyme. This suggests that the integrity of the beta-lactam ring is essential for inactivation. The inactivation was not reversed after

Inactivation of yeast glucose-6-P dehydrogenase by pyridoxal-P and penicillin G

| Enzyme treatment | Relative activity[a] Before dialysis | After dialysis[b] |
|---|---|---|
| Untreated control | 100 | 94.6 |
| Treated with 0.5 mM pyridoxal-P for 5 min[c] | 0 | 91.5 |
| Treated with 40 mM penicillin G for 1 h[c] | 12.3 | 10.8 |
| Treated with 0.5 mM pyridoxal-P for 5 min and then with 40 mM penicillin G for 1 h | 0 | 82.7 |

[a] The enzyme activity was based on the initial rate measured according to the procedure previously described[5]. The relative activity of 100 is equivalent to the sp. act. of 285 units per mg protein. [b] The reaction mixture (4 ml) was dialyzed at 4 °C for 8.5 h against 800 ml of 50 mM Tris-HCl buffer (pH 6.6) containing 1 mM cysteine (3 buffer changes). [c] Treatment of the enzyme (12 µg/ml) with pyridoxal-P or penicillin G was carried out in 50 mM Na borate buffer (pH 8.0).

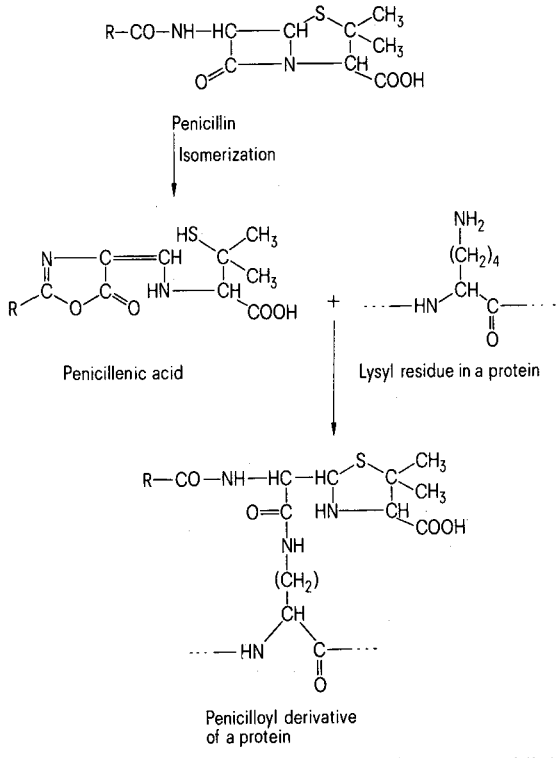


Figure 1. Proposed mechanism for the formation of N-acyl linkage between penicillin and the ε-amino group of lysyl residue in protein (from Levine[4]).

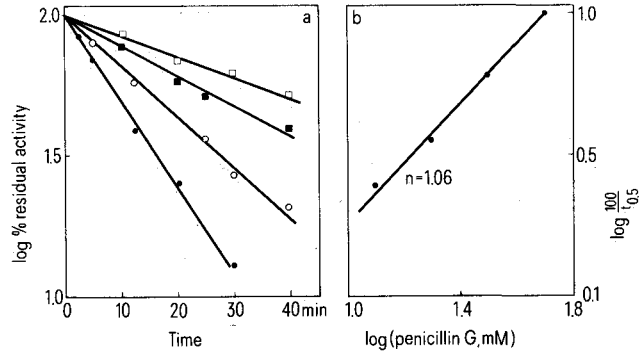


Figure 2. *a* Rate of inactivation of yeast glucose-6-P dehydrogenase with varying concentrations of penicillin G. Enzyme. (12 µg/ml) was incubated in 50 mM Na borate buffer (pH 8.0) at 25 °C with Na salt of penicillin G at 12.5 mM (□), 20 mM (■), 32 mM (○), or 50 mM (●). Aliquots were removed at the times shown and immediately assayed for enzyme activity. The activity of the enzyme incubated under the identical conditions but without penicillin G remained constant throughout the experimental period. *b* Determination of the order of the enzyme inactivation with respect to penicillin G.

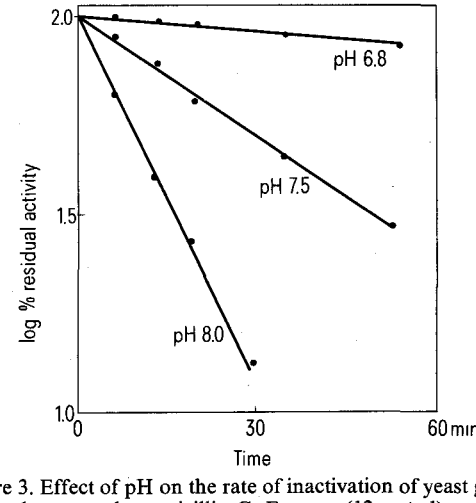


Figure 3. Effect of pH on the rate of inactivation of yeast glucose-6-P dehydrogenase by penicillin G. Enzyme (12 µg/ml) was incubated at 25 °C with 50 mM penicillin G in 50 mM Na borate buffer at different pH. Aliquots were removed at the times indicated and immediately assayed for enzyme activity. The activity of the enzyme incubated at different pH but without penicillin G remained constant throughout the experimental period.

removal of virtually all penicillin G by extensive dialysis or by treatment with 1 N $NH_2OH$ for 1 h at pH 7.0. Since penicillin is potentially an acylating agent of protein[12], the irreversible inactivation by penicillin G may result from the acylation of the essential functional group of the enzyme molecule. It is considered that N-acyl linkage, rather than S-acyl linkage or O-acyl linkage, might have been formed since the latter would be expected to be reversed by $NH_2OH$[13]. We recently observed that yeast glucose-6-P dehydrogenase was extremely sensitive to inactivation by pyridoxal-P[5]. Since pyridoxal-P is highly specific for modifying $\epsilon$-amino group of lysine in proteins[6], it is considered that this enzyme contains reactive lysyl residues at its active site. The reaction of pyridoxal-P with amino groups is well known to result in the formation of Schiff base which is reversible upon dilution or dialysis[10]. The data presented in the table show that inactivation of this enzyme by pyridoxal-P was reversed by dialysis. These data also show that in the presence of pyridoxal-P the irreversible inactivation of the enzyme by penicillin G was largely prevented. This suggests that pyridoxal-P and penicillin G may react with the enzyme at the same site, namely by acylating the $\epsilon$-amino group of a lysyl residue essential for catalytic activity. The enhanced rate of inactivation with increasing pH appears to be consistent with the proposed mechanism, since according to this mechanism the unprotonated form of the amino group should react with penicillin G.

Although it is generally regarded that penicillenic acid is probably the molecular form that acylates proteins[4,12], it is still considered possible that penicillin may directly acylate protein without molecular rearrangement. This is based on the assumption that beta-lactam carbonyl is reactive and susceptible to nucleophilic attack as originally suggested by Woodward[14] who recognized that inhibition of amide resonance by ring strain could account for this.

1 This work was supported by grant RR-8006 from the General Research Branch, Division of Research Resources, NIH (USA).
2 L. Weinstein, in: The Pharmacological Basis of Therapeutics, p. 1132. Ed. L.S. Goodman and A. Gilman. Macmillan, New York 1975.
3 D.J. Tipper and J.L. Strominger, Proc. natl Acad. Sci. USA *54*, 1133 (1965).
4 B.B. Levine, J. exp. Med. *112*, 1131 (1960).
5 P.F. Han, G.Y. Han, H.C. McBay and J. Johnson, Experientia *36*, 1149 (1980).
6 G. Colombo and F. Marcus, Biochemistry *13*, 3085 (1974).
7 H.M. Levy, P.D. Leber and E.M. Ryan, J. biol. Chem. *238*, 3654 (1963).
8 M.C. Scrutton and M.F. Utter, J. biol. Chem. *240*, 3714 (1965).
9 F. Marcus, S.M. Schuster and H.A. Lardy, J. biol. Chem. *251*, 1775 (1976).
10 M. Milhausen and H.R. Levy, Eur. J. Biochem. *50*, 453 (1975).
11 B. Aurebekk and C. Little, Int. J. Biochem. *8*, 757 (1977).
12 A. Goldstein, L. Aronow and S.M. Kalman, Principles of Drug Action: The Basis of Pharmacology, p. 497. Wiley, New York 1974.
13 G.J. Roth and C.J. Siok, J. biol. Chem. *253*, 3782 (1978).
14 R.B. Woodward, in: The Chemistry of Penicillin, p. 440. Ed. H.T. Clarke, J.R. Johnson and R. Robinson. Princeton University Press, Princeton, N.J., 1949.

## Acetaldehyde metabolism in *Drosophila melanogaster*[1]

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*Summary.* A quantitative study on in vitro acetaldehyde degradation in homogenates from *Drosophila melanogaster* flies shows that aldehyde oxidase plays the major part in acetaldehyde detoxification. However, in a strain, called 'AO null', because its *Aldox* locus produces no aldehyde-oxidase, acetaldehyde is also degradated by a still unknown mechanism. Alcoholdehydrogenase which is responsible for the dehydrogenation of ethanol in acetaldehyde, appears to catalyze the reversed reaction as well, regenerating ethanol from acetaldehyde.

In *Drosophila melanogaster* alcohol dehydrogenase (ADH) seems to play an essential part in the ethanol tolerance[2-6], the physiological and ecological importance of which is no longer questioned[7-10]. It has generally been accepted[6] that *Drosophila* ADH converts ethanol into acetaldehyde, a highly toxic substance[11], which therefore must be transformed immediately into non toxic and even useful products. As a matter of fact, it has been shown that ethanol is used as a food in metabolism[12-14].

Our purpose is to question the part played by the enzyme aldehyde oxidase (AO) in the detoxification of acetaldehyde. Experiences in vivo have shown that a mutant strain 'AO null' which does not produce any active AO is nevertheless tolerant of alcohol[15], although it is not very tolerant of acetaldehyde[11]. According to David[15] such a physiological paradox could probably be explained by the discovery of another locus which produces a small amount of AO even when the *Aldox* locus (3-56.7) does not give active AO. This small amount of enzyme should be sufficient for the detoxification of the metabolic acetaldehyde produced by ADH, although unable to cope with greater quantities of acetaldehyde in food and environment.

The same problem is here approached by another way. One measures the degradation in vitro of small quantities of acetaldehyde by homogenates of flies from three strains, one of which is 'AO null' whereas another is 'ADH null' but with normally active AO. The third strain, having highly active ADH and AO enzymes, is used as a control[11].

The flies were grown on axenic medium, according to David and Clavel[16] and 'deyeasted' before the test, according to Day et al.[17]. Immediately before homogenization, they were immobilized by cooling. For each experiment, 500 mg of adult flies were homogenized with a Potter homogenizer in 1.1 ml of 0.25 M sucrose Tris buffer at pH 7.5. The homogenate was centrifugated at 15,000 rpm for 20 min, at 0 °C.

60-µl aliquots of supernatant were then placed in 5-ml vials, with 20 µl 20 mM acetaldehyde, 10 µl 0.05 M Tris buffer (pH 7.5) and, when indicated, the following compound: pyrazole 20 mM + sodium azide 1 mM Tris buffer. The vials were then sealed hermetically and incubated for 1 h at 15 °C.

5-ml vials were also incubated containing 60 µl 0.25 M sucrose Tris buffer, 20 µl acetaldehyde 20 mM and 10 µl