thus confirms the results already gained from the equation (2).

Discussion. The method used seems to be sufficient for our purpose, though O'Donnell[9] took exception to it because it does not limit the contact of the calcium solution to the mucosa alone. However, kinetic studies[8] in the normal rat led to the conclusion that the saturable component for slice influx could be ascribed mainly to transport across the mucosal surface. The similar estimate of linear component P of Ca influx across only the mucosal or the serosal surfaces reflects a simple diffusion into tissues. In this study, analysis of this linear component does not show change of P in any case. In both the Cd-exposed and the 1.25 $(OH)_2D_3$-treated control group, the apparent increase of J_2 is not confirmed by statistical analysis. This fact may be due to the complexity of the system under study and experimental values dispersion. Administration of 1.25 dihydroxyvitamin D_3 to exposed animals restores Jm to a value comparable to that in control and 1.25 $(OH)_2D_3$ groups. Furthermore the experiments show a peculiar effect of 1.25 dihydroxyvitamin D_3 on the half-saturation constant Kt which is increased in all groups 1.25 $(OH)_2D_3$ dosed. These results express the double impact of this vitamin D_3 metabolite[10] on the turn-over activity of the carrier decreased by Cd^{++} intoxication, and on the affinity of the carrier in all cases. Little is known about the mechanism of an action of 1.25 dihydroxyvitamin D_3 on the intestinal absorption. To De Luca[11] its appears to be similar to the action of classic steroid hormones on their target tissue. Active calcium transport decrease in cadmium-intoxicated rats may result from a direct calcium-cadmium interaction[12] on the intestinal calcium carrier, or from an inactivation of 25 $(OH)D_3$ 1-α-hydroxylase in the kidney[13].

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Inactivation of yeast glucose-6-P dehydrogenase by aspirin[1]

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Summary. Glucose-6-P dehydrogenase is irreversibly inactivated by treatment with Na salts of aspirin. Kinetic data show that 1 molecule of aspirin reacts with each active unit when the enzyme is inactivated. The rate of inactivation is enhanced with increasing pH but is reduced in the presence of glucose-6-P or $NADP^+$. Na salicylate fails to inactivate the enzyme.

Although aspirin (acetylsalicylic acid) has been known to acetylate numerous biological macromolecules for more than a decade[2], studies on the functional consequences of treating enzymes with aspirin have largely been neglected with the exception of recent interest in prostaglandin synthetase[3]. We recently showed that the sensitivity of a fructose-1,6-bisphosphatase to allosteric inhibition by adenosine-5'-monophosphate was irreversibly modified after treatment with aspirin[4]. In this communication we report the irreversible inactivation of a glucose-6-P dehydrogenase (EC 1.1.1.49) by aspirin.

Materials and methods. Aspirin, crystalline glucose-6-P dehydrogenase from baker's yeast (270 units/mg of protein), and other chemicals used in this study were all purchased from Sigma. The activity of glucose-6-P dehydrogenase was assayed at 25 °C by measuring the rate of $NADP^+$ reduction at 340 nm. The standard reaction mixture (1 ml) contained 50 mM Tris-HCl buffer (pH 7.5), 0.25 mM $NADP^+$, 2 mM $MgCl_2$, 0.6 mM glucose-6-P, and an appropriate amount of glucose-6-P dehydrogenase. Reactions were initiated by addition of glucose-6-P dehydrogenase. Treatment of the enzyme (12 µg/ml) with different concentrations of Na salt of aspirin was carried out at 25 °C in 70 mM Tris-HCl buffer at the pH-values indicated in the figure and the table legends. Aliquots were removed at the times specified and immediately assayed for enzyme activity.

Results and discussion. Treatment of yeast glucose-6-P dehydrogenase with Na salt of aspirin resulted in rapid inactivation of the enzyme. The results obtained with various concentrations of aspirin are presented in figure 1,A, as semilogarithmic plot. The inactivation reaction follow pseudo-first order kinetics until about 90% of the enzyme had been inactivated, and prolonged incubation led to complete inactivation. The inactivation reaction

Effect of pH on the inactivation of yeast glucose-6-P dehydrogenase by aspirin*

pH	Half-time for inactivation ($t_{0.5}$) (min)
8.5	2.3
8.0	4.5
7.5	8.9
7.0	29.3
6.5	62.4
6.0	184.3

*Enzyme (12 µg/ml) was incubated at 25 °C with 10 mM Na salt of aspirin in 70 mM Tris-HCl buffer at the pH-values indicated. Portions were removed at specific times and assayed for enzyme activities as described in materials and methods. Times for 50% inactivation ($t_{0.5}$) were determined from semilogarithmic graphs in which residual activity was plotted against time as in figure 1,A.

between this enzyme and aspirin can be represented by the following equation: $E+nI \xrightarrow{k_1} EI_n$, where E represents the free enzyme, I the inactivator (aspirin), n the number of molecules of aspirin, and EI_n enzyme-aspirin complex. The rate of this reaction can be written as: $-d(E)/dt = k_1(E)(I)^n$. Since the aspirin concentration greatly exceeds that of the enzyme, the pseudo-first order rate constant, k', equals $k_1(I)^n$. Taking logarithms give: $\log k' = \log k_1 + n\log(I)$. Therefore, a plot of log k' against log(I) should give a straight line with a slope equal to n, where n is the number of molecules of aspirin reacting with each active unit of the enzyme to produce an inactive-aspirin complex. For convenience k' can be replaced by the reciprocal of the half-time for inactivation ($t_{0.5}$) without affecting the slope. When the data from figure 1,A, were plotted in this manner, a slope of 0.95 was obtained (figure 1,B). This suggests that the reaction of 1 molecule of aspirin per active unit of the enzyme is necessary for inactivation. This method was first introduced by Levy et al.[5] and subsequently used by many others[6–9].

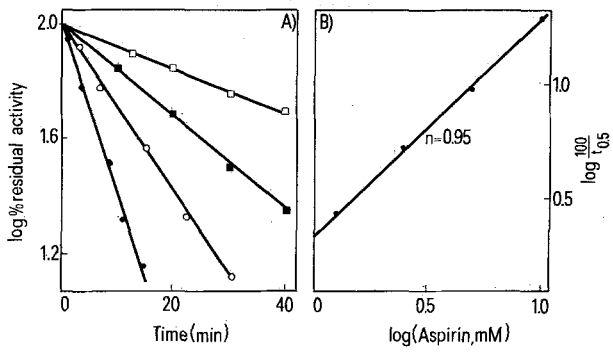

Fig. 1. *A* Rate of inactivation of yeast glucose-6-P dehydrogenase with varying concentrations of aspirin. Enzyme (12 µg/ml) was incubated in 70 mM Tris-HCl buffer (pH 7.8) at 25 °C with Na salt of aspirin at 1.25 mM (□), 2.5 mM (■), 5 mM (○), or 10 mM (●). Aliquots were removed at the times shown and immediately assayed for enzyme activities. The activity of the enzyme incubated under the identical conditions but without aspirin remained constant throughout the experimental period. *B* Determination of the order of the enzyme inactivation reaction with respect to aspirin. The data are plotted as log of the reciprocal of $t_{0.5}$ for inactivation against log of aspirin concentration. The slope of the plot gives n=0.95.

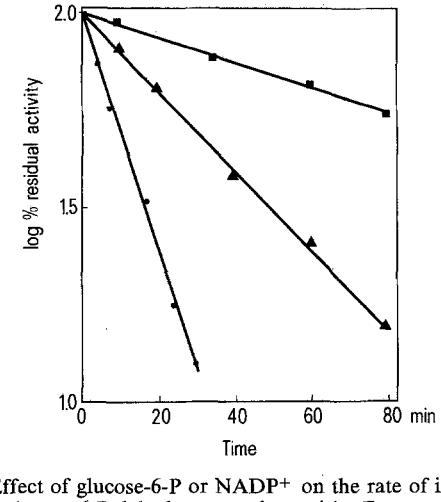

Fig. 2. Effect of glucose-6-P or $NADP^+$ on the rate of inactivation of yeast glucose-6-P dehydrogenase by aspirin. Enzyme (12 µg/ml) was incubated in 70 mM Tris-HCl buffer (pH 7.8) at 25 °C with 5 mM Na salt of aspirin. Aliquots were removed at the times shown and immediately assayed for enzyme activity. The following symbols signify: (■) incubation in the presence of 0.2 mM glucose-6-P; (▲) incubation in the presence of 0.2 mM $NADP^+$; (●) incubation without any addition.

As shown in the table, the rate of inactivation by aspirin increased greatly with increasing pH over the range (6.0–8.5) studied. The half-time for inactivation by 10 mM aspirin incubated at pH 6.0 was 184 min as compared with only 2.3 min when incubated at pH 8.5. The rate of inactivation decreased markedly when treatment with aspirin was performed in the presence of glucose-6-P or $NADP^+$. On molar basis glucose-6-P was more effective than $NADP^+$ in protecting the enzyme against aspirin inactivation (figure 2). The inactivation of this enzyme by aspirin was not reversed after removal of virtually all aspirin by extensive dialysis. Incubation of the inactivated enzyme (after dialysis) with 1 M NH_2OH at pH 7.4 for 1 h failed to restore the activity. Treatment of this enzyme with 10 mM Na salicylate at pH 7.8 for 2 h was not found to inactivate the enzyme. These observations strongly suggest that inactivation of this enzyme by aspirin may result from the acetylation of the functional group essential for catalytic activity.

We have also investigated the sensitivity of this enzyme to inactivation by pyridoxal-P. It was found that incubation of this enzyme with 0.5 mM pyridoxal-P at pH 7.8 for 5 min followed by reduction with $NaBH_4$ according to the procedure described by Colombo et al.[10] resulted in almost complete irreversible inactivation of the enzyme. Since pyridoxal-P is a chemical modification reagent with high specificity for lysine[11], it is considered that, like glucose-6-P dehydrogenase from *Leuconostoc mesenteroides*[6], the enzyme from Bakers yeast may also contain lysine at its active site. Aspirin is known to acetylate the ε-amino groups of lysyl residues in serum albumin and hemoglobin[12,13]. The fact that the inactivation of this enzyme is not reversed by NH_2OH suggests that the inactivation may result from the formation of N-acetyl linkage[3]. Assuming that the lysyl ε-amino group is acetylated, then the unprotonated form should react with aspirin. In such case the rate of inactivation will be expected to enhance with increasing pH, and this is exactly what we have observed. We are now investigating whether aspirin and pyridoxal-P inactivate the enzyme by modifying the same site of the enzyme molecule.

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