and in the very lysine-rich histone F1 (ref. [2,3]), the moderately lysine-rich histone F2b, studied in our laboratory, shows a similar assymetry. Most of the neutral and hydrophobic amino acids are situated in the central part of this protein (i.e., peptides T3 or T11, T16, and T21 contain 31 of the 94 nonbasic residues present in F2b histone). Regions on either side of the nonbasic center are relatively enriched in basic amino acids with the NH_2 terminal region being definitely more basic (14 of the 33 residues are basic amino acids) than the COOH terminal portion (12 of the 54 amino acids are basic residues). The biological significance of the observed assymetry in amino acid distribution in the F2b histone from calf thymus is not clear[13].

Zusammenfassung. Die Aminosäuresequenz der Histonfraktion F2b aus Kalbsthymus wird angegeben und die Verteilung der basischen Aminosäuren als nicht symmetrisch ermittelt.

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Stereospecificity of the Prostaglandin 15-Dehydrogenase from Swine Lung[1]

Recent studies of the metabolism of natural prostaglandins (PGs) have revealed a number of significant pathways commencing with either (1) saturation of the C-13 double bond[2], (2) β-oxidation of the carboxylic acid side chain[3,4], or (3) dehydrogenation at C-15 producing 15-keto PGs[2,5]. Of these, shortening of the carboxylic acid side chain[6] or oxidation of the hydroxy group at C-15 (present in all primary PGs) causes a marked decrease in smooth muscle stimulating, vasodepressor[7], and platelet aggregation inhibition properties[8].

The 15-dehydrogenase from the high speed supernatant of swine lung is the only isolated PG metabolizing enzyme with a single chemical action. Anggård and Samuelsson[5] report that this enzyme effects 15-dehydrogenation of all PGs except those of the PGB type. The enzyme is NAD dependent and highly specific for PGs – even 15(S)-hydroxyeicosa-8,11,13-trienoic acid is inert[5].

Diastereomeric PGs and a wide variety of non-acidic prostane derivatives are now available by efficient total syntheses[9–12] and a preliminary examination of the activity of these substances on 4 different pharmacological preparations has revealed unexpected biological potency for some of the unnatural diastereomers[13]. This communication is a preliminary account of the study of the stereostructural requirements (for substrate) of the swine lung dehydrogenase using synthetic substrates with the object of determining whether structure modifications would reduce the rate of biological degradation without reducing the pharmacological activity and thus serve as an indication of the modifications needed to produce substances having long-lasting actions like those of the natural PGs.

Materials and methods. The swine lung 15-hydroxyprostaglandin dehydrogenase was isolated by a slight modification of the published method[5]. Dehydrogenation rates with *nat*-PGE_1[14] (Km = 5.3 μM) were comparable to those reported (5.6 μM)[5]. The formation of 15-keto-prostanes was followed spectrophotometrically using the 340 nm absorption of the NADH generated in the reaction[5]. In the case of PGE_1-diastereomers the amount of 15-keto-PGE_1 formed could be confirmed spectrophotometrically using the strong, but transient, absorption that develops at 500 nm on base treatment. In addition the presence of the 15-keto products was confirmed by thin-layer chromatographic (TLC)[15] comparison with authentic samples prepared by chemical oxidation (using dicyano-dichlorobenzoquinone).

Results and discussion. Typical time courses of the enzymic dehydrogenation of *nat*-PGE_1 and the 4 racemic isomers are shown in Figure 1[16]. The maximum ΔA_{340} values obtained are generally 75–95% of those expected based on the generation of a stoichiometric amount of NADH, and are proportional to the amount of *nat*-PGE_1 used over a 10-fold range in concentration (5–66 μM). The amount of NADH decreases slowly after the maximum value has been reached, suggesting the presence of a NADH-dependent enzyme system (and substrate?) since the amount of 15-keto-PGE_1 does not diminish in the manner of the 500 nm absorption. For this reason direct assays of 15-keto-PGE_1 are more reliable for substrates reacting slowly.

Figure 1 clearly indicates that racemic $PG(E\alpha\alpha)_1$ and $PG(E\beta\alpha)_1$ are dehydrogenated at half molar amounts of *nat*-$PG(E\alpha\alpha)_1$ indicating that only one antipode is used.

[1] This work was supported by grants from the U.S. National Institutes of Health to Harvard University and by O.N.R. and N.I.H. grants to the Worcester Foundation for Experimental Biology.

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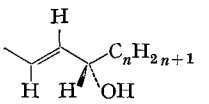
[16] The nomenclature employed for PG diastereomers is an extension of the present nomenclature in which the configurations, α or β, of hydroxyl groups at C-9, C-11, and C-15 are indicated in parentheses, see ref. [15].

Prolonged incubation with excess enzyme preparation did not lead to reaction beyond 50%. The recovered PG(E$\alpha\alpha$)$_1$ and PG(E$\beta\alpha$)$_1$ from preparative incubations showed positive Cotton effects centered at ~295 nm indicating the enantio-structure[17]. Thus neither the optical specificity nor the rate are altered with the change in configuration at C-11.

The racemic 15-epimer of PGE, *rac*-PG(E$\alpha\beta$)$_1$, was not dehydrogenated by the enzyme, however it does inhibit the reaction of *nat*-PG(E$\alpha\alpha$)$_1$. A Lineweaver-Burk plot (Figure 2) indicated competitive inhibition[18]. The dissociation constant of the enzyme-inhibitor complex was ~13 μM. Thus it appears that 15-*epi*-PGE$_1$ complexes effectively, but non-productively, with the enzyme. Comparison of the observed dissociation constant and the Michaelis constant for *nat*-PGE$_1$ suggests that the 15(S)-hydroxyl group is a binding site as well as the site of chemical reaction. Final confirmation of this conclusion will require inhibition studies using resolved PG(E$\alpha\beta$)$_1$.

If the immediate neighborhood of the 15-hydroxyl group is important in binding one might expect to find reaction with compounds containing the grouping shown below, in a prostaglandin-like molecule differing greatly in other parts of the molecule.



This expectation has been realized in the case of *rac*-PG(E$\beta\beta$)$_1$. Figure 1 shows a small, but significant, reaction with this stereoisomer. Longer incubations indicated that this reaction occurs at *ca.* 15% of the rate observed with natural PGE$_1$ and proceeds to 50% completion. The ORD of the recovered PG(E$\beta\beta$)$_1$ indicated that *ent*-PG(E$\beta\beta$)$_1$ had been preferentially consumed by the enzyme[17]. *Enantio*-PG(E$\beta\beta$)$_1$ has the unnatural back-bone configuration, but the proper 15(S)-hydroxyl configuration. Racemic PG(E$\beta\beta$)$_1$ has previously been shown to be a potent substance on some smooth muscle preparations (2–10 times as active as racemic PGE$_1$)[13], and this activity was tentatively ascribed to the *enantio* form. This conclusion has been confirmed in part by the synthesis of partially resolved *ent*-PG(E$\beta\beta$)$_1$, optical purity ~35%,

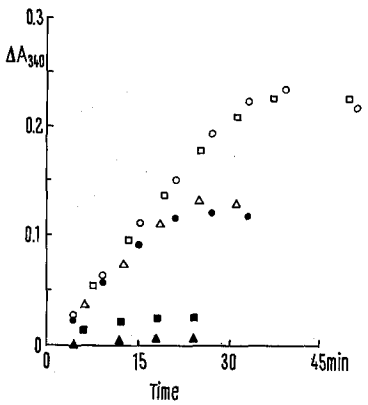


Fig. 1. Time course of NADH formation for the incubation of ca. 10 units of swine lung dehydrogenase with 0.05–0.2 μmoles of substrate, 2 μmoles of NAD, 4 μmoles of reduced glutathione and 3 μmoles EDTA in 3 ml of 0.1 M of potassium phosphate buffer (pH 7.4) at 42 °C. ○, 0.1 μmole *nat*-PGE$_1$; ●, 0.05 μmole *nat*-PGE$_1$; □, 0.2 μmole *rac*-PGE$_1$; △, 0.1 μmole *rac*-PG(E$\beta\alpha$)$_1$; ▲, 0.2 μmole (*rac*-PG(E$\alpha\beta$)$_1$; ■, 0.2 μmole *rac*-PG(E$\beta\beta$)$_1$.

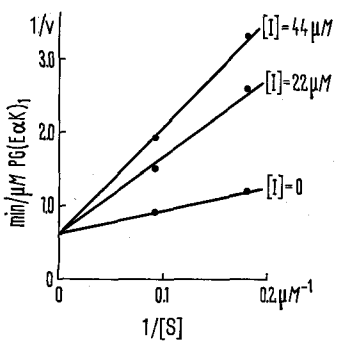
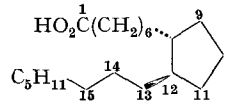
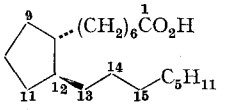


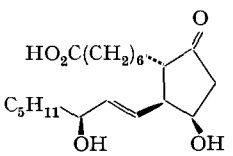
Fig. 2. Inhibition of the dehydrogenation of *nat*-PG(E$\alpha\alpha$)$_1$, by *rac*-PG(E$\alpha\beta$)$_1$. A Lineweaver-Burk plot of 15-keto-PGE$_1$ formation-rates (estimated from ΔA_{500} on base treatment). Enzyme (~20 units), 2 μmoles NAD, 4 μmoles reduced glutathione, 9 μmoles of EDTA, and *nat*-PG(E$\alpha\alpha$)$_1$ were incubated at 42 °C in 9 ml of 0.1 M potassium phosphate buffer (pH 7.4) with 0, 0.2, or 0.4 μmole of racemic PG(E$\alpha\beta$)$_1$. [I] indicates the concentration of *rac*-PG(E$\alpha\beta$)$_1$, as an inhibitor. K_m = 5.3 μM, $K_i \simeq$ 13 μM.



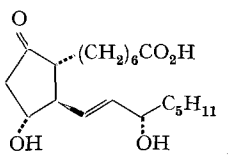
ent-prostanoic acid



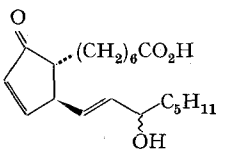
nat-prostanoic acid



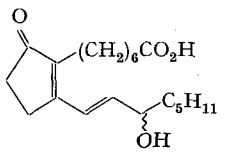
antipode of 11,15-epi-PGE$_1$



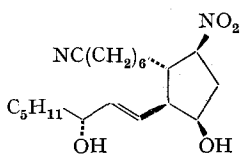
natural-PGE$_1$ = *nat*-PG(E$\alpha\alpha$)$_1$ = *ent*-PG(E$\beta\beta$)$_1$



PGA-types



PGB-types



ent-9β-nitro-11β,15α-dihydroxy-*trans*-13-prostenonitrile

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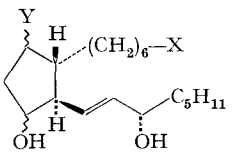
which is at least as potent as *rac*-PG(E$\beta\beta$)$_1$ on the rat uterus. The synthesis proceeds from *enantio*-9β-nitro-11β, 15α-dihydroxy-*trans*-13-prostenonitrile (recovered from an incubation of the racemic compound with the swine lung dehydrogenase) by steps already outlined[10].

Some comments on the relationship between smooth muscle stimulating activity and dehydrogenase activity appear warranted. A 15(S)-hydroxyl group appears to be the major stereostructural requirement for both. The dehydrogenase system is less sensitive to the stereochemistry at C-11 whereas smooth muscle stimulating activity appears to be enhanced markedly by buttressing hydroxyl groups (formally *cis* at C-11 and even C-9, unpublished data) and only slightly affected by the back-bone stereochemistry in the most favorable cases (*ent*-PG(E$\beta\beta$)$_1$ as an example). This difference in sensitivity to the back-bone stereochemistry (inversion at C-8 and C-12) has allowed us to partially realize our original objective. Thus pure *ent*-PG(E$\beta\beta$)$_1$ should be at least as potent as *nat*-PGE$_1$ on the rat uterus and far more potent on other tissue preparations but would be degraded at only 15% of the rate of *nat*-PGE$_1$.

Finally the dehydrogenase accommodates major changes at positions remote from C-15 and C-12. The functionality present at C-1, C-9 and C-11, as well as the configuration of these groups, can be varied within the limits shown below without altering the rate of the enzymic reaction substantially. The 9-formamidoprostanes are not dehydrogenated, apparently due to a greatly reduced affinity for the enzyme – they show no inhibitory effect on the reaction of *nat*-PGE$_1$[19], [20].



X = –CO_2H, –CN
Y = =O, –OH, –NH_2, –NO_2
Y ≠ –NHCHO

Zusammenfassung. Die stereochemischen Voraussetzungen der Prostaglandin-15-Dehydrogenase aus Schweinelunge werden im Hinblick auf die pharmakologische Aktivität anhand einer Reihe synthetischer Prostaglandin E$_1$-Präparate abgeklärt.

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20 October 1969.

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[20] The authors are grateful to the technical assistance of Miss Anne Marie Plasse.

Endocrine Cells in Mammalian Gastric Mucosa: Possible Storage Sites for Vitamin B_{12}-Binding Proteins

Very little is known of the cellular localization of the gastric antipernicious principle (the intrinsic factor, IF). The first cell to be implicated as the source of IF was the argentaffin (enterochromaffin) cell[1–3]. This hypothesis fell into disrepute because of a poor correlation between the distribution of these cells and of IF in many species[4]. In the rat, the chief cell has been proposed as the storage site for IF[5]. It is quite evident from the literature that in several other species the chief cell is not the cellular storage site for IF[6–8] and both the oxyntic cell and the pyloric gland cell have been advocated. Current opinion appears to locate IF to different cells in different species[8, 9]. From pure speculation it appears likely that a compound with such a specific function as IF should be produced and stored in the same type of cell in all species. Recently, it was reported that in the rat the regional and topographical distribution of vitamin B_{12}-binding proteins, associated with IF activity, coincided with the distribution of a system of histamine-containing enterochromaffin-like cells[10], which are believed to have an endocrine function[11–16]. The enterochromaffin (argentaffin) and enterochromaffin-like (argyrophil, non-argentaffin) cells have the morphological characteristics of polypeptide- or protein-secreting endocrine cells, and it has been suggested that they are active in the formation and secretion of gastrointestinal polypeptide hormones[11–16]. Enterochromaffin cells are recognized by their intense yellow formaldehyde-induced fluorescence, which reflects their content of 5-HT[17]. Enterochromaffin-like cells are devoid of 5-HT but can be demonstrated in L-DOPA-treated animals by their green formaldehyde-induced dopamine fluorescence[17]. Enterochromaffin cells usually retain their yellow fluorescence also after L-DOPA treatment, which

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