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The reduction of prostaglandin E_2 to prostaglandin $F_{2\alpha}$ by various animal tissues

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The conversion of prostaglandin E_2 (PGE_2) to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) by an enzyme in sheep red blood cells has recently been described (Hensby, 1974). This type of metabolism, namely reduction of the 9-oxo group, has previously only been described in a few instances. These include guinea-pig liver and urine (Hamberg & Samuelsson, 1969; Hamberg & Israelsson, 1970), various rat organs (Leslie & Levine, 1973), human urine (Hamberg & Wilson, 1972) and baker's yeast (Schneider & Murray, 1973). This type of metabolism is of interest because of the many markedly different pharmacological actions of PGE_2 and $PGF_{2\alpha}$. The metabolism of PGE_2 by tissue homogenates of heart, liver and kidney from a variety of animal species, namely guinea-pig, rabbit, horse, sheep, dog and pig have now been studied.

Tissues were removed and used as soon as possible after death (being stored on ice if transport required). Homogenates were prepared by chopping the tissues into small pieces and homogenizing in 3 volumes of ice-cold 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 2,400 *g* for 30 min and the resulting supernatant was carefully decanted off. This was incubated at 37°C with PGE_2 at a substrate concentration of 14 μM (sp. act. 35.2 Ci/mole) and NAD (10 mM) plus NADH (10 mM) for 2 hours.

The products of the incubation were converted to methyl esters after extraction and separated on Lipidex 5000 gel columns of 29-30 ml bed volume (Brash & Jones, 1974). The eluting solvent was a heptane, chloroform mixture 80 : 20 (v/v) and the room temperature 20 ± 1°C. Fractions (3.0 ml) were collected and the aliquot assayed by scintillation counting to enable the elution profile of the column to be obtained. Where $PGF_{2\alpha}$

appeared to be a metabolic product, the experiment was repeated at a ten-fold increased level of substrate. In addition, [^{14}C]-PGE_1 (at the same substrate concentration) was also compared in parallel with the PGE_2.

Following column chromatography further evidence of identification was obtained by thin layer chromatography in two solvent systems. In addition the trimethylsilyl ether methyl ester derivative of the material eluted in the $PGF_{1\alpha}$ zones had a retention time for ^{14}C corresponding to authentic [^{14}C]-$PGF_{1\alpha}$ on radio gas chromatography.

Conclusive evidence of identification for both $PGF_{1\alpha}$ and $PGF_{2\alpha}$ was obtained by combined gas liquid chromatography mass spectrometry.

Of the tissues studied, only the livers of guinea-pig, rabbit and horse and heart of the horse have been found to produce the corresponding PGF_α on incubation with PGE. At no time has any evidence for the production of the corresponding PGF_β been obtained. In addition PGE has been found to be metabolized to products other than the PGF_α in all tissues so far studied. These other metabolites have provisionally been identified as the corresponding 13,14-dihydro PGE, 13,14-dihydro 15-oxo PGE and 15-oxo PGE.

To date the majority of experiments have been performed using rabbit liver homogenates and the results indicate that there is a wide range of 9-oxo reductase activity within the liver of any one species. In rabbit liver the yield of $PGF_{2\alpha}$, formed from PGE_2, has ranged from 25 to 48% of the total radioactivity recovered.

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Biological activity of prostaglandin D_2 on smooth muscle

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The 9,11-cyclic endoperoxide formed during prostaglandin (PG) biosynthesis can be converted to either PGE, PGF_α or PGD (Fig. 1) (Granström, Lands & Samuelsson, 1968; Nugteren & Hazelhof, 1973; Hamberg & Samuelsson, 1973). It has been reported that PGD_1 and PGD_2, in contrast to PGE_1, possess negligible biological activity (Nugteren & Hazelhof, 1973).

Our initial experiments indicated that PGD_2 is active on smooth muscle. We have therefore compared PGD_2 with PGE_2 and $PGF_{2\alpha}$ upon a variety of biological preparations, a number of which are known to give qualitatively different responses to E and F type prostaglandins.

On preparations in which PGE_2 is a more powerful inhibitor than $PGF_{2\alpha}$ (equipotent molar ratio 300 to 3,000). PGD_2 was less active than PGE_2 but two to four times more active than $PGF_{2\alpha}$. Such relative activities were found on cat tracheal muscle *in vitro*, dog hind limb vessels *in vivo* and rabbit oviduct *in vivo*.

In the sheep, PGD_2, like $PGF_{2\alpha}$, is pressor whereas PGE_2 is depressor. PGD_2, however, is 20 to 140 times ($n = 5$) more active than $PGF_{2\alpha}$, producing effects at threshold doses from 0.4 to 20 ng/kg. These pressor responses were not abolished by phenoxybenzamine hydrochloride (3 mg/kg). On the sheep hind limb, perfused at constant flow, the pressure was increased by PGD_2 injected intra-arterially. In the rabbit, PGD_2 was