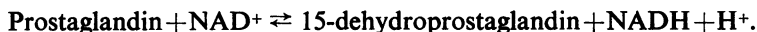


### Enzymatic assay of the prostaglandins

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We recently described the purification and properties of a 15-hydroxy prostaglandin dehydrogenase from swine lung (Änggård & Samuelsson, 1966). This enzyme catalyses the oxidation of the secondary alcohol group at C-15 to a ketone in all known prostaglandins with the exception of those containing the dienone structure absorbing at 278 nm (PGB-compounds and their 19-hydroxylated analogues). The enzyme, which is present in the soluble fraction of a homogenate, uses NAD<sup>+</sup> but not NADP<sup>+</sup> as cofactor. The reaction can thus be written as follows:



Because the equilibrium favours the oxidation of prostaglandin, it is evident that the enzyme can be used in an enzymatic assay method for the prostaglandins based on the measurement of either of the products formed. Such a method would combine the selectivity of the enzyme with the high sensitivity attainable with fluorimetric measurements of pyridine nucleotides.

In the present communication the general principles for the enzymatic analysis of prostaglandins are discussed. Using direct fluorimetric measurement of the generated NADH, 10<sup>-10</sup>–10<sup>-8</sup> moles of prostaglandins have been assayed. By coupling the prostaglandin dehydrogenase catalysed reaction to an amplifying system (Lowry, Passoneau, Schultz & Rock, 1961), the sensitivity of the assay is further increased to a lower limit of about 10<sup>-12</sup> moles.

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### N<sup>1</sup>-Methylnicotinamide uptake by rat kidney slices: reversible and irreversible inhibition

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The identification and isolation of protein components of complex systems has become theoretically feasible through use of site-directed irreversible inhibitors, but has been demonstrated in only a few cases. We are applying this technique to the isolation of proteins involved in the transport of N<sup>1</sup>-methylnicotinamide (NMN) by kidneys.

Slices of cortex from kidneys of rats were incubated with <sup>14</sup>C-N<sup>1</sup>-methylnicotinamide (NMN) and <sup>3</sup>H-p-aminohippurate (PAH) in Robinson's kidney medium for 30 min. The slices were analysed for radioactivity, the uptakes of NMN and PAH computed and corrected for 25 % extra-cellular space. Each result was the average of two determinations. Doses of different inhibitors were added to the system and the change in uptake of NMN and PAH (expressed as % inhibition) was compared with controls.

With a set of homologues of NMN, N<sup>1</sup>-alkylnicotinamides (alkyl groups of 2 to 11 carbons) the inhibition of NMN uptake increased with length of alkyl chain from 2 to 6 carbons, did not change from 6 to 9 carbons, and dropped nearly to zero with the 11-