EFFECTS OF PHOTOLYSABLE AZIDO-ANALOGUES OF ADENOSINE, AMP AND ADP ON HUMAN PLATELETS. N.J. Cusack and G.V.R. Born, Department of Pharmacology, University of Cambridge, Cambridge, England.

The aggregating effect of ADP on human platelets and the inhibiting effect of adenosine are apparently mediated by different receptors on the cells' outer membranes (N.J. Haslam & G.M. Rosson, 1975, *Molec.Pharmacol.*, **11**, 528). Photolysable azido-analogues of adenosine & ADP have been prepared so that the receptors for them can be labelled. 2-Azidoadenosine inhibits adenosine deaminase competitively before and irreversibly after irradiation at 365 nm (N.J. Cusack and G.V.R. Born, 1976, *Proc.Roy.Soc.B*, **193**, 307). 2-Azidoadenosine inhibited platelet aggregation by ADP more than adenosine itself and increased platelet cAMP as effectively as did adenosine or 2-chloroadenosine. 2-Azido AMP inhibited aggregation much more effectively than AMP itself or than 2-chloro AMP. 2-Azido ADP was about 5 times more potent than ADP in causing aggregation. All these 2-azido derivatives were photolysable by irradiation at 365 nm which did not affect platelet functions.

THE NUMBER AND NATURE OF ADP RECEPTORS ON HUMAN BLOOD PLATELETS DETERMINED WITH A PHOTOAFFINITY LABEL. D.E.Macfarlane and D.C.B.Mills. Specialized Center for Thrombosis Research, Temple University Hospital, Philadelphia, Pa., U.S.A.

ADP interacts with platelets to cause shape change, aggregation and inhibition of adenylate cyclase. Previous attempts to measure the binding of ADP to its receptor on intact platelets have been frustrated by the low ratio of specifically bound ADP to the ADP trapped in centrifuged pellets. Several 2-substituted derivatives of ADP have higher apparent affinities for the ADP receptor, improving this ratio. We have prepared 2-azido 5'-ADP from 2-chloroadenosine in 20% yield. It was more active than ADP as an antagonist of PGE₁-induced elevation of cyclic AMP ($K_i = 96$ nM, cf ADP $K_i = 760$ nM) and also as an inducer of shape change and aggregation. 2-Azido [$\beta^{32}\text{P}$]-5'-ADP (2.5 Ci/mmol) was prepared and used to study binding. Platelets were separated from plasma by centrifugation through silicone oil (1.023 gm/ml) in a modified Eppendorf centrifuge. Scatchard plots of the binding data were resolved into two linear components, one of zero affinity, corresponding to trapped plasma, measured independently with [^{14}C] sucrose, and a high affinity component with apparent $K_D = 110$ -160 nM. There were 400-700 of these binding sites per platelet. 2-Azido 5'-ADP is photolysable with light at <310 nm, forming a reactive nitrene potentially suitable for photoaffinity labelling. 2-Azido [$\beta^{32}\text{P}$]-5'-ADP was photolysed in the presence of washed platelets which were subsequently rewashed, solubilized and electrophoresed on SDS-polyacrylamide gradient slabs. Several peaks of radioactivity were observed in the high molecular weight region of the gel (ca 100-250 kD) one of which was less labelled in the presence of excess ADP or ATP to block access of the label to the receptor.