tryptophan/ml and rose linearly during the perfusion to reach a concentration of 4.6 ± 0.2 μg/ml at 60 minutes.

At the end of a 60 min perfusion and when 0.1 mM tryptophan was perfused the mean concentration of kynurenine was 6.64 μg/g liver (wet weight) and when 1.0 mM tryptophan was in the medium the final mean concentration of kynurenine in the medium was 31.1 μg/g liver. Pretreatment of the rats with hydrocortisone (5 mg/kg) 3 h before the start of the perfusion resulted in a threefold increase of pyrrolase activity as measured *in vitro* and increased the concentration of kynurenine in the medium at the end of the 60 min perfusion to 14.45 ± 2.82 μg/g when 0.1 mM tryptophan was perfused and 145 ± 13.3 μg/g when 1.0 mM tryptophan was perfused.

When allopurinol (20 mg/kg) was injected with the hydrocortisone (5 mg/kg) and also added to the 0.1 mM tryptophan medium (4 mg allopurinol /100 ml medium) it totally inhibited the hydrocortisone-induced rise in kynurenine concentration in the medium. The rise in pyrrolase activity (measured *in vitro*) produced by hydrocortisone injection was totally inhibited by the simultaneous injection of allopurinol (20 mg/kg).

The disappearance of tryptophan from the medium did not always reflect the activity of pyrrolase measured either *in vitro* or by the appearance of kynurenine in the medium. This is in agreement with the observations of Kim & Miller (1969). Unlike these authors, however, we find that hydrocortisone pretreatment increases subsequent kynurenine production by the isolated perfused rat liver. Furthermore, we observed that changes in pyrrolase activity measured *in vitro* were of the same order as the changes in kynurenine concentration in the perfusion medium.

References

CURZON, G. & GREEN, A.R. (1969). Effects of immobilization on rat liver tryptophan pyrrolase and brain 5-hydroxytryptamine metabolism. *Br. J. Pharmac.*, **37**, 689-697.

GREEN, A.R., WOODS, H.F., KNOTT, P.J. & CURZON, G. (1975). Factors influencing effect of hydrocortisone on rat brain tryptophan metabolism. *Nature (Lond.)*, **255**, 170.

HEMS, R., ROSS, B.D., BERRY, M.N. & KREBS, H.A. (1966). Gluconeogenesis in the perfused rat liver. *Biochem. J.*, **101**, 284-292.

KIM, H.G. & MILLER, L.L. (1969). The functional significance of changes in activity of the enzymes tryptophan pyrrolase and tyrosine transaminase after induction in intact rats and in the isolated perfused rat liver. *J. Biol. Chem.*, **244**, 1410-1416.

WOODS, H.F., EGGLESTON, L.V. & KREBS, H.A. (1970). The cause of hepatic accumulation of fructose-1-phosphate on fructose loading. *Biochem. J.* **119**, 501-510.

Role of platelet aggregation in bronchoconstriction in guinea-pigs

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Non-steroidal anti-inflammatory drugs (NSAID) prevent bronchoconstriction due to bradykinin (B) (Collier & Shorley, 1960), ATP and ADP (Collier, James & Schneider, 1966), arachidonic acid (AA) (Berry, 1966) and slow reacting substances (Berry & Collier, 1964; Vargaftig, Miranda & Lacoume, 1969). Since some of these agents cause platelet aggregation which is inhibited by NSAID, we tested the hypothesis that bronchoconstriction by B, by AA and by ADP or ATP are mediated by platelet release reaction occurring when clumps are trapped in lung vessels. This possibility was investigated in pentobarbitone anaesthetized guinea-pigs prepared for recording of bronchial resistance to inflation (Konzett-Rössler method) and of carotid blood pressure. Acetylcholine (5-20 μg kg^{-1} i.v.) was injected 3-5 times, until constant responses were obtained, after which other drugs were given. Platelet counts were made on arterial blood, and aggregation was studied by standard techniques (Born & Cross, 1963; Vargaftig, Tranier & Chignard, 1974) on guinea-pig platelet rich plasma obtained from citrated blood. Anti-platelet plasma (APP) was prepared by injecting thrice washed broken guinea-pig platelets in complete Freunds adjuvant into both fore paws of a rabbit. Two further s.c. injections were made at weekly intervals, blood was collected in citrate on the 21st day. APP lysed guinea-pig platelets placed on the aggregometer, which was inhibited by Na_2EDTA (2.5 mM) but unaffected by indomethacin (0.5 mM). Lyophilized APP was reconstituted to its initial volume with 0.9% NaCl (w/v) and injected slowly (1 h) i.v. into the guinea-pig (1 ml kg^{-1}).

Circulating platelets decreased 62 ± 4.8%

(mean ± s.e. mean) 10 s after ADP (100 $\mu g\ kg^{-1}$; n = 22) administration. One minute later the drop was 21 ± 7 and the count returned to basal levels within 5-10 minutes. Higher doses of ATP (0.5-1 $mg\ kg^{-1}$) were required for similar effects. AA (500 $\mu g\ kg^{-1}$) decreased the number of platelets by 70% ± 4.4 (n = 11). Bronchoconstriction and the platelet decrease were suppressed by 10 $mg\ kg^{-1}$ of aspirin i.v. B (0.5-1 $\mu g/kg^{-1}$) had no effect on platelet counts. Platelet depletion resulted in suppression of ADP- and of ATP-induced bronchoconstriction, whereas the effect of B and of AA was maintained or only slightly reduced. Administration of propranolol (5 mg/kg^{-1} + 2 mg/kg^{-1} i.v.) restored responses to B demonstrating that the reduction of its effect was due to antagonism by released adrenaline and not to platelet depletion (Collier, 1966). B failed to aggregate guinea-pig platelets in presence of the kininase inhibitor BPP9A (250 $\mu g/ml^{-1}$), whereas ATP induced a marked aggregation, probably via conversion to ADP. In conclusion, agents likely to start the release reaction and thus to activate prostaglandin synthesis (Zucker & Peterson, 1968; Smith, Ingerman, Kocsis & Silver, 1974) with formation of bronchoconstrictor substances (Vargaftig & Dao Hai, 1972a) interact with different sites, all of them subject to blockade by aspirin. In the case of ADP and of ATP, this site is circulating platelets. Bradykinin probably induces formation of bronchoconstrictor substances by activating release of prostaglandin precursors directly from the lungs (Vargaftig & Dao Hai, 1972b).

References

BERRY, P.A. (1966). Slow reacting substance in anaphylaxis (SRS-A): Its release, actions and antagonism, Thesis for Ph.D., London' Council for National Academic Awards.

BERRY, P.A. & COLLIER, H.O.J. (1964). Bronchoconstrictor action and antagonism of a slow reacting substance from anaphylaxis of guinea-pig isolated lung. *Br. J. Pharmac.*, **23**, 201-216.

BORN, G.V.R. & CROSS, M.J. (1963). The aggregation of blood platelets. *J. Physiol. (Lond.)* **168**, 178-195.

COLLIER, H.O.J. (1966). Self-antagonism of bronchoconstriction induced by bradykinin and angiotensin. In *Hypotensive Peptides*, ed. Erdös, E.G., Back, N. & Sicuteri, F., pp. 305-313. Berlin: Springer Verlag.

COLLIER, H.O.J., JAMES, G.N.L. & SCHNEIDER, C. (1966). Antagonism by aspirin and fenamates of bronchoconstriction and nociception induced by adenosine-5'-triphosphate. *Nature (Lond.)* **212**, 411-412.

COLLIER, H.O.J. & SHORLEY, P.G. (1960). Analgesic antipyretic drugs as antagonists of bradykinin. *Br. J. Pharmac.*, **15**, 601-610.

SMITH, J.B., INGERMAN, C., KOCSIS, J.J. & SILVER, M.J. (1974). Prostaglandins in Platelet Function. *Thromb. Res.*, **4**, 49-55.

VARGAFTIG, B.B. & DAO HAI, N. (1972a). Paradoxical inhibition of the effects of bradykinin by some sulfhydryl reagents. *Experientia*, **28**, 59-62.

VARGAFTIG, B.B. & DAO HAI, N. (1972b). Selective inhibition by mepacrine of the release of 'rabbit aorta contracting substance' evoked by the administration of bradykinin. *J. Pharm. Pharmac.*, **24**, 159-161.

VARGAFTIG, B.B., MIRANDA, E.P. & LACOUME, B. (1969). Inhibition by nonsteroidal anti-inflammatory agents of *in vivo* effect of 'slow reacting substance C'. *Nature (Lond.)*, **222**, 883-885.

VARGAFTIG, B.B., TRAINER, Y. & CHIGNARD, M. (1974). Inhibition by sulfhydryl agents of arachidonic acid-induced platelet aggregation and release of potential inflammatory substances. *Prostaglandins*, **8**, 133-156.

ZUCKER, M.B. & PETERSON, J. (1968). Inhibition of adenosine diphosphate-induced secondary aggregation and other platelet functions by acetylsalicyclic acid ingestion. *Proc. Soc. exp. Biol. Med.*, **127**, 547-551.

The effect of sulphinpyrazone, sodium aspirin and oxprenolol on the formation of arterial platelet thrombi

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Platelets play a major role in the formation of arterial thrombi (see Mustard, Kinlough-Rathbone & Packham, 1974). In an effort to study the reaction *in vivo*, various methods of injuring the microcirculation have been examined to produce platelet thrombosis (white body formation) (see Didisheim, 1972).

In the present study we have used the hamster cheek pouch as described by Duling, Berne & Born (1968) and perfused with a modified Krebs solution at 5 ml/min as described by Duling & Staples (1974). The following parameters were found to be optimal for producing white body formation. After a 30 min equilibration period, a glass micropipette with a 3 μm tip filled with 1 M KCl was placed in contact at approximately 90° with the wall of an arteriole of 40-60 μm