morphine for further investigation of these interesting changes.

Our results are obviously of only a preliminary nature and leave unanswered the question of the fate of the 94–97 per cent of the morphine activity not accounted for. Clearly there are many ways in which the newly formed substances could be translocated, such as within the ramifying latex system or by transfer to the phloem and xylem vessels of the vascular bundles. Dispersal by these means to other parts of the plant might account for some of the missing activity. Some of the newly formed substances might be broken down into $^{14}CO_2$, for which, unfortunately, we were not able to test under the conditions of the experiment. In further experiments we intend examining the newly formed compounds as soon as possible after their formation in the latex.

Interest in the seeds stems from our earlier observation that there is no anatomical connexion between the latex vessels and the seeds[11]. Latex vessels occur in all parts of the plant except the stamens, ovules and seeds; alkaloids are also absent from these organs. The presence of derivatives of morphine in the seeds is therefore of some interest.

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Significance and Regulation of Methaemoglobinaemia

Beutler and Mathai[1] have rather simplified my thesis[2]. A central theme, that the methaemoglobin–oxyhaemoglobin and oxidized-reduced glutathione (GSSG–GSH) systems are loosely linked in a buffering system against the selective and destructive effects of different types of oxidizing compound, has already been discussed[3]. On the basis of published[4–10] and otherwise unpublished[2] observations, it was also proposed that the pentose phosphate pathway governs the equilibrium between methaemoglobin formation and GSH depletion, and thus generally between the degrees of methaemoglobinaemia and haemolysis in erythrocytes exposed to catalytic oxidants. The suggestion was made that, by providing a source of reductant energy which varies in degree and direction with the oxidant stress, the pentose phosphate pathway might well have played an important part in the evolution of the anucloar mammalian erythrocyte. These concepts, relating to the adaptive role of the pentose phosphate pathway, seemed essentially in accord with previous and concurrent observations concerning the role and control of the pentose phosphate pathway in other mammalian tissues (see refs. 11–22, and refs. 70–74 cited by Jandl *et al.*[23]).

Of more immediate relevance were the differences observed *in vitro*, which appeared sufficiently striking to warrant the following tentative classification of oxidant haemolysins[2]: (1) 'simple' oxidants such as nitrite, which cause methaemoglobin formation, with GSH depletion only if the pentose phosphate pathway is inadequate, and irreversible damage in normal cells only after prolonged exposure; (2) catalytic oxidants such as quinone, *p*-aminophenol and the phenylhydrazines, which cause in succession GSH depletion, methaemoglobin formation, oxidative destruction of haemoglobin, and osmotic hyperfragility, and which are relatively ineffective stimulants of NADPH-dependent methaemoglobin reduction; (3) catalytic oxidants such as naphthoquinones, which catalyse methaemoglobin formation and GSH depletion in normal cells potently and to a similar relative degree, cause irreversible damage only after gross GSH depletion, and are powerful catalysts of NADPH-dependent methaemoglobin reduction; (4) powerful catalysts of NADPH-dependent methaemoglobin reduction, such as methylene blue, which cause gross GSH depletion (and frank haemolysis *in vivo*[24]) only with an inadequate pentose phosphate pathway of GSSG reduction; (5) catalytic oxidants such as 2-naphthol and 'Primaquine', which selectively increase osmotic fragility relative to other changes; (6) aromatic compounds such as phenacetin and acetylsalicylic acid, which presumably oxidize erythrocytes only after conversion *in vivo* to an active derivative; (7) aromatic compounds such as 'Primaquine', which are active *in vitro*, but appear likely to exert an additional effect after degradation *in vivo*[24].

Because acetylphenylhydrazine causes complete loss of GSH before achieving methaemoglobin formation, prior formation of methaemoglobin by nitrite would not be expected to favour GSH depletion by this aromatic compound. The experiments of Beutler and Mathai[1] thus seem in agreement, rather than in disagreement, with my thesis. Clearly, their findings neither confirm nor refute the buffering effect of the oxyhaemoglobin-methaemoglobin system on the GSH–GSSG system in erythrocytes exposed to more potent methaemoglobin-forming catalysts, such as naphthoquinones, or to 'simple' oxidants such as nitrite. However, our observation that methaemoglobin formation usually precedes both haemoglobin destruction and osmotic hyperfragility suggests that the production of methaemoglobin may buffer the cell against irreversible oxidative changes, even by drugs which have first depleted GSH. The very conversion of oxyhaemoglobin to a reconvertible derivative, incapable of catalysing the generation of erythrotoxic hydrogen peroxide by oxidants[25,26], might well be regarded as a cellular adaptive mechanism.

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