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The major intestinal metabolites of anthocyanins are unlikely to be conjugates of their parent compounds but metabolites of their degradation products

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Anthocyanins are a class of flavonoid that impart blue and red colour to many berries and fruits[1,2]. It has been reported that those individuals in the population who consume the highest amounts of anthocyanins are at lower risk of developing CHD and CVD relative to the lowest consumers[3]. Moreover, numerous *ex vivo* and *in vitro* experimental studies have described vascular mechanisms of action that are in keeping with protection against CVD[4–13]. However, doses of anthocyanins utilised in these studies are commonly >10 μM[5,7,8], which despite a high dietary consumption of anthocyanins (doses >500 mg in many clinical studies[4,14,15]) does not appear achievable in human subjects. Furthermore, the *in vitro* mechanistic bioactivity of anthocyanins has been exclusively explored using aglycones and glycoside conjugates, despite a lack of evidence establishing these compounds as the biologically-available forms.

As spontaneous degradation of anthocyanins to phenolic acids and aldehydes is reported to occur under experimental[16] and biological conditions[17,18], it is likely that degradation products of anthocyanins contribute substantially to their alleged benefits. Thus, the overall objective of the present study was to establish the chemical fate of anthocyanins and the nature of the breakdown process in the gut.

The Caco-2 cell-culture studies indicated that after 4 h incubation of anthocyanins in cell-culture media (cell-free Dulbecco's modified Eagle's medium (DMEM)) 43 % of the initial level of cyanidin-3-glucoside (C3G) and 2 % of that of cyanidin remains ($P<0.0001$). The parent anthocyanidin structure spontaneously degrades to yield protocatechuic acid (PCA) and phloroglucinaldehyde (PGA), which is confirmed in two other tested matrices (phosphate and Hank's buffers). In intestinal epithelial cell cultures (Caco-2 cells) the degradation product PCA is metabolised to sulfate and glucuronide conjugates, as indicated by both enzyme hydrolysis (sulfatase and glucuronidase treatment) and MS (m/z; PCA 155, sulfate 235, glucuronide 331; cyanidin 287, sulfate 367, glucuronide 463). All values are expressed relative to cell-free incubations, controlled for temperature, time, pH and extraction procedure across nine replicates for each of DMEM and Hank's buffer.

It is difficult to establish whether PGA contributes equally to the metabolite pool as its recovery in cultured cell media and cell-free DMEM is extremely low (13 % and 26 % respectively; $P<0.0001$). Additionally, the exact extent of sulfation and glucuronidation is difficult to establish as treatment with sulfatase and glucuronidase resulted in deglycosylation of C3G, and subsequent degradation of the aglycone results in the formation of new degradation products. It is, however, clear that degradation and recovery are major concerns in anthocyanin analysis.

These data suggest that the major intestinal metabolites of anthocyanins are unlikely to be conjugates of the parent compounds, but metabolites of their degradation products. Thus, efforts to establish the biological activities of anthocyanins must be re-established using the phenolic acid and aldehyde products of degradation, along with their respective metabolites.

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