We have been interested in the phylogenetic development of this enzyme, the bilirubin conjugation pathway. Studies of salmon BVR activity in various species of fish and mammals have been reported and have been employed to examine the relationship of BVR to the mammalian enzyme. The purification of BVR from a number of mammalian sources (ox [2], rat [3], pig [4] and human [5]) has been successful in birds, reptiles and amphibians. There has been some question as to the absence of this enzyme in fish, which is considered to be a toxic metabolite in phyla that produce it while other higher vertebrates apparently function successfully in its absence. However, there is increasing evidence that bilirubin may play a significant role as an antioxidant [1].

The purification of BVR from salmon liver homogenate was undertaken in buffer A (20mM Tris/HCl, pH 7.2, 150mM NaCl, 1mM EDTA) and after centrifugation at 13,600 g for 30 minutes the supernatant was subjected to ammonium sulfate fractionation (40-70%). This material was dialysed against three changes of buffer B (10mM ammonium phosphate pH 7.2; 100mM KCl). After centrifugation at 20,000 g for 10 minutes the supernatant was loaded onto a Sephacryl S-200 column (120 x 4.5 cm) and eluted with the same buffer. This material was diluted 1:1 with 10mM sodium phosphate buffer, pH 7.2 and then applied to a column of 2',5'-ADP-Sepharose. The enzyme was eluted using a 0.05-1.0M KCl gradient (2 by 200ml). This material was used for kinetic studies; to raise monospecific polyclonal anti-serum in NZW rabbits; and to generate peptides for amino acid sequencing.

Fig. 1 shows the results of DEAE-cellulose chromatography. The enzyme reproducibly elutes as two peaks, with a possible third peak forming a shoulder late in the second peak (Fig. 1). All further purification work was carried out using the second major peak (P2). BVR eluted as a single peak on S-200 gel filtration, corresponding to a relative molecular mass of 34,000. After 2'S ADP-Sepharose chromatography, the enzyme was homogeneous on SDS-PAGE with a Mr of 34,000 (Fig. 2).

Preliminary sequence determination on cyanogen bromide fragments has presented evidence that the salmon enzyme shares significant relatedness to the rat enzyme [6] (although this is not reflected in immunological activity). In Fig. 3 the rat sequence [7] is shown and fragments that have been tentatively identified as revealing partial identity are marked. Salmon BVR appears to be structurally related to the rat enzyme which raises some interesting phylogenetic questions as to the enzyme is not reported to exist in amphibians, birds or reptiles. Further work is necessary to confirm the latter claim, however it raises the possibility that the BVR gene may exist in the CNS where the pigment is neurotoxic, leading to kernicterus. While the enzyme is present in mammals and some species of fish, it is apparently absent from birds, reptiles and amphibians. However, there is increasing evidence that bilirubin may play a significant role as an antioxidant [1].