Short Communications

Binding of tryptophan and iron by reptilion plasma proteins

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Plasma proteins of reptiles constitute from three to seven per cent of blood (Dessauer 1974). Studies with gel and other electrophoretic systems have shown that these proteins are a complex mixture with molecular weights in the range comparable to those of mammals (Roberts & Seal 1965). Dessauer (1974) states, that although some fractions of reptile plasma proteins have electrophoretic mobilities comparable to the albumin and α, β and γ globulins of human plasma proteins, the specific proteins which are present in fractions of comparable mobility are often different for species of the two classes. Therefore, electrophoretic results should be interpreted with care and comparable proteins should be studied first by a variety of techniques before homogeneity is assumed.

Some plasma proteins of reptiles have specialized transport functions. Albumin of the alligator *(Alligator mississippiensis)* and other reptiles binds, amongst other ions, tryptophan (McMenamy & Watson 1968) and transferrin binds iron (Barber & Sheeler 1963). Multiple transferrins are present in the plasma of many reptiles (Dessauer *et al* 1962) and the albumin region of the electrophoretogram of certain species of snake may also show more than one prominent band (Hattingh & Willemse 1976). By studying the binding of tryptophan and iron to the plasma proteins insight may thus be gained as to the number of transferrins and albumins present in the blood of a given species with the ability to bind these ions. This approach was used in the present study to investigate the albumins and transferrins of puff-adders and crocodiles (*Bitis arietans* and *Crocodylus niloticus*). The puff-adder exhibits four closely associated protein fractions in the albumin region when studied on 7½% polyacrylamide gels (Hattingh & Willemse 1976). In the case of the crocodiles, plasma from different aged animals were obtained to see what changes, if any, occur in trytophan and iron binding with age.

Puff-adders were obtained from the Hartebeespoort Snake and Animal Park. Blood was drawn by cardiac puncture and mixed with heparin (1 mg/ml). Crocodile plasma was obtained from a farm in Rhodesia from animals aged six months, 18 months, 3½ years and 5½ years. Initial characterization of the plasma proteins was carried out on both 5 and 7½% polyacrylamide gels in 0.02M Tris-Glycine buffer at pH 8.6 applying 2.5μl plasma to the gels (Hattingh 1972). Plasma protein concentration was determined with a biuret method using bovine serum albumin as a standard (Lane 1957).

Results obtained by subjecting plasma to electrophoresis on 7½% polyacrylamide gels are shown in Fig. 1. The transferrin and albumin regions are indicated by T and A respectively, and represent areas of the gel most closely corresponding to albumin and transferrin areas in human blood. It is evident that crocodile plasma displayed one albumin band and the puff-adder three. Crocodile plasma showed one transferrin band and puff-adder two and there seems to be very little change with age in crocodile blood. Except for the additional band marked X in the blood of the 3½ year specimen, the gels were very similar. The same results were found on 5% gels, except that puff-adder plasma now showed only two bands in the albumin region. Plasma protein concentration in the case of the puff-adder was estimated at 6.38 ± 1.54 g% ($N = 12$) and that of the crocodiles at 3.90 ± 0.64 g% ($N = 4$, mean $\pm$ S.D.). Due to the fact that only one sample was available for each age crocodile, this value represents the pooled data.

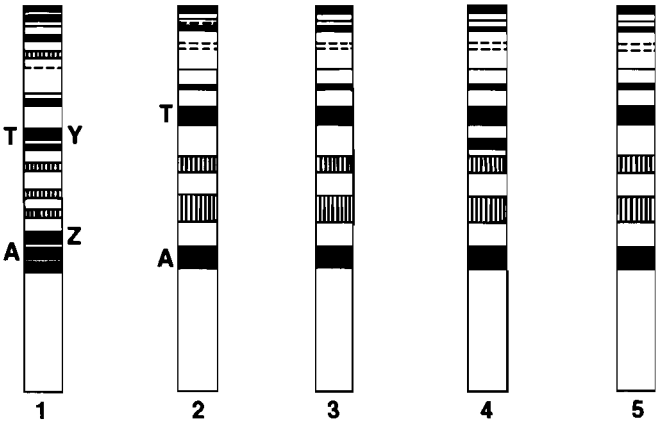

Fig. 1 Diagramatic representation of results obtained by subjecting plasma to electrophoresis on 7½% polyacrylamide gels at pH 8.6. Anode towards the bottom. 1-puff-adder, 2-6 month old crocodile, 3-18 month old crocodile, 4-3½ year old crocodile and 5-5½ year old crocodile. A-albumin region and T-transferrin region. See text for explanation of X,Y and Z.

Albumin in plasma was labelled with tryptophan by incubating 1 ml with 10μl of ^{14}C-tryptophan (Radiochemical Centre, Amersham; specific activity 252μ Ci/mg) for one hour at room temperature. Radioactivity was determined after electrophoresis of 2.5μl (7½% gels) by slicing frozen gels into 2 mm sections, incubating the sections in 0.4 ml 30% (V/V) H_2O_2 at 90° for one hour and finally adding 10 ml of Aquagel (Chemlab, P.O. Box 56218, Pinegowrie, S.A.). Samples were counted in a

Packard scintillation counter, model 3390. The results obtained showed that puff-adder plasma carried tryptophan only in the topmost of the three "albumin bands", and crocodile blood only in the albumin band (fraction marked with Z in puff-adder plasma in Fig. 1). No change with age was observed in crocodile blood.

Transferrin in plasma was labelled with $^{59}FeCl_3$ (Radiochemical Centre, Amersham; specific activity 3mCi/mg) according to the methods of Wenn and Williams (1968). In all cases only a single peak was observed after counting gel slices (see above). The protein fractions responsible for iron binding are indicated with Y in puff-adder plasma, and T in crocodile plasma in Fig. 1. Again no change was observed with age in crocodile blood.

The results obtained in the present study again show that care should be taken when comparing reptilian plasma protein fractions with those of the human. Assuming similar properties for reptilian and mammalian transferrins and albumins (Dessauer 1974) it would seem that both species investigated have only one transferrin fraction although two prominent bands are found in the puff-adder in this region after electrophoresis. In the case of albumin, puff-adder plasma merits further study seeing that only one fraction binds tryptophan. It is possible that other "transferrin" fractions and "albumins" in the case of the puff-adder may eventually be found to bind iron and tryptophan under different conditions of study as what was used here. In this regard it is known that the two transferrins of *Pseudemys scripta* have different heat stabilities and unload complexed iron at different acidities (Barber & Sheeler 1963). Due to the fact that the method used to label transferrin with iron in the present study involves adding the iron in O.1M HCl, it is highly unlikely that conditions were unfavourable for iron labelling by another species of transferrin if it were present. In the case of reptilian albumin it is known that this molecule binds tryptophan less strongly than its counterpart in avian and mammalian blood (Dessauer 1974). Here again, however, prolonged incubation with tryptophan did not change our results and it would then seem that only one of the "albumins" of the puff-adder has the ability to bind this molecule. It remains to be seen whether the two associated fractions are indeed "albumins".

Finally the results obtained with the plasma of the different aged crocodiles indicated that no major change occurs in plasma protein fractions with age (in the range used for the present study). It also appears as if only one transferrin and one albumin are present in these animals (taking the above arguments into account). It would be interesting to compare the above data to that of local crocodiles of the same species to investigate any possible geographical differences which are known to occur in some reptilian species (Dessauer 1974). This may yield further information concerning the adaptability of these animals and possible hybridization.

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References

BARBER, A.S. & SHEELER, P. 1963. A comparative study on the iron-binding properties of vertebrate blood sera. *Comp. Biochem. Physiol.* 8: 115-122.

DESSAUER, J. 1974. Plasma proteins in Reptilia. In *Chemical Zoology* eds Florkin, M. & Scheer, B.T. Vol. 8. Academic Press: New York. p. 187-216.

DESSAUER, H., FOX, W. & HARTWIG, Q. I. 1962. Comparative study of transferrins of Amphabia and Reptilia using starch-gel electrophoresis and autoradiography. *Comp. Biochem. Physiol.* 5: 17-29.

HATTINGH, J. 1972. Observations on the blood physiology of five South African freshwater fish. *J. Fish Biol.* 4: 555-563.

HATTINGH, J. & WILLEMSE, G.T. 1976. Hematological observations on the puff-adder, *Bitis arietans* (Ophidia: Viperidae). *Herpetol.* 32: 245-247.

LANE, E. 1957. Methods in Enzymology, Vol. 3. Academic Press: New York, p. 450-451.

McMENAMY, R.H. & WATSON, F. 1968. Indole-albumin association: a comparative study. *Comp. Biochem. Physiol.* 26: 332-335.

ROBERTS, R.C. & SEAL, U.S. 1965. Sedimentation analysis of vertebrate serum proteins. *Comp. Biochem. Physiol.* 16: 327-331.

WENN, R.V. & WILLIAMS, J. 1968. The isoelectric fractionation of hens egg ovotransferrin. *Biochem. J.* 108: 69-74.