STEROIDAL REGULATION OF VITELLOGENIN mRNA LEVELS IN THE LIVER OF THE PAINTED TURTLE CHRYSEMYS PICTA.

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Vitellogenin (vtg) in the major yolk protein produced by the liver of oviparous vertebrates and its production is regulated by ovarian steroids. In a previous report we described the isolation of several cDNA clones encoding part of two different vitellogenin proteins in the painted turtle Chrysemys picta (Charnock-Jones et al., Bull. MDIBL 32: 139-140, 1993). Here we describe experiments which investigate the interaction of the steroids estrogen and progesterone in controlling the levels of vitellogenin mRNA in the liver of C.picta.

Liver tissue from steroid treated turtles (both intact males and ovariectomized females) was collected and stored at -70°C until needed. RNA was prepared using the acid phenol extraction method of Chomczynski and Sacchi (Anal Biochem 162: 156-159, 1987). Total RNA (30µg) was analyzed by gel electrophoresis and northern blotting. The probes used were fragments of the C.picta vtg cDNAs already described. These probes, although they do not cross hybridize using the conditions used in this study, gave indistinguishable results.

Figure 1 shows northern blot analysis of liver RNA from male (A) and ovariectomized female (B) turtles treated with the steroids designated. A single hybridizing band of approximately 6.5kb can be seen. This size is in good agreement with that reported in other species. (for example 6.6kb in the trout Le Guellec et al., Gen.Comp.Endocrin 71: 359-371 1988). In both males and ovariectomized females (Fig 1A vs 1B) estradiol markedly induced vtg mRNA (not seen in either of the controls); in males P appeared to reduce the amount of vtg mRNA, compared to E alone (lane E vs E+P) and P alone had no effect. In ovariectomized females, progesterone appeared to synergise with E, thus increasing vtg mRNA further, and P alone appeared to increase vtg mRNA above the control level. It is not possible to say whether these differences are due to the different endocrine milieu of males vs ovariectomized females prior to treatment or simply due to animal to animal variation. However, it does indicate that as in other species E induces vtg mRNA, and it suggests that P may be either a positive or a negative regulator, depending upon the preexisting endocrine milieu. This is in keeping with observations that E and P may have either synergistic or antagonistic effects on protein synthesis; further tissue specific expression of progesterone receptor isoforms A and B may be involved, with PR-A being a general inhibitor of transcription, and PR-B stimulatory (Vegeto et al., Molec.Endo.7:1244-1255 1993).

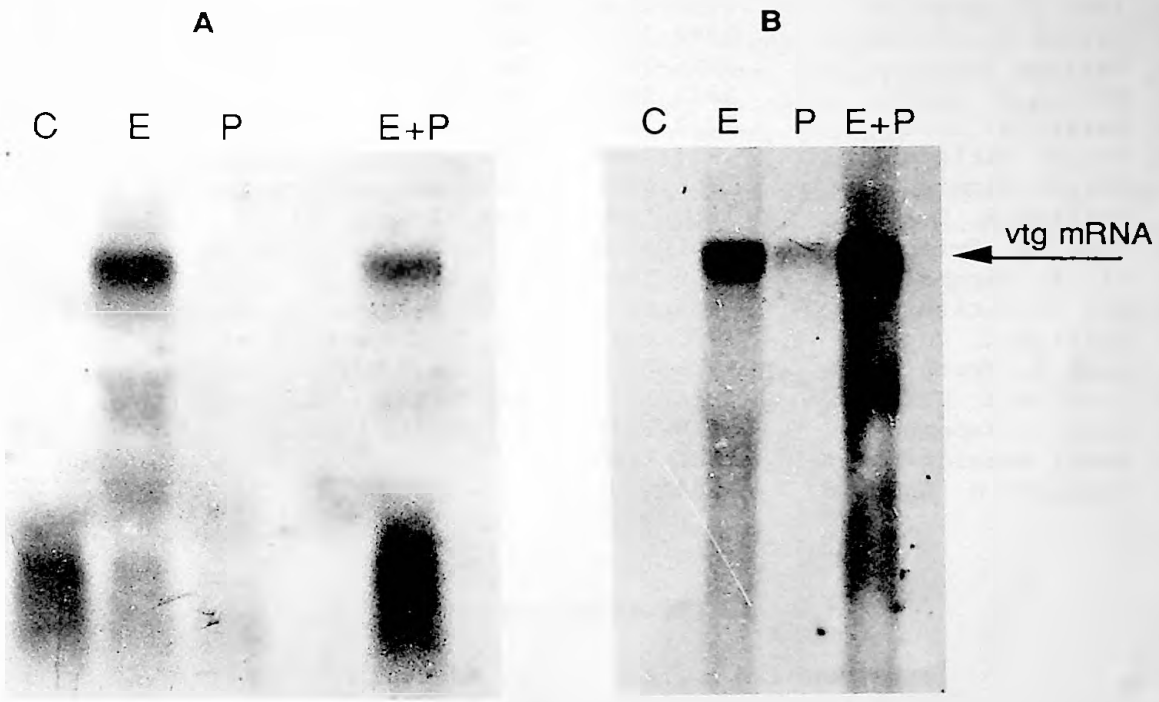

Fig 1
Northern blots of RNA prepared from the liver of male (A) and ovariectomized female (B) turtles. Animals were treated with the steroids estrogen (E), progesterone (P), or a combination of both. (C) designates untreated control. Blots were hybridized with a turtle vitellogenin probe and washed at high stringency. Blots were exposed to film for 60hr.

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