Biotin-binding proteins in eggs of oviparous vertebrates

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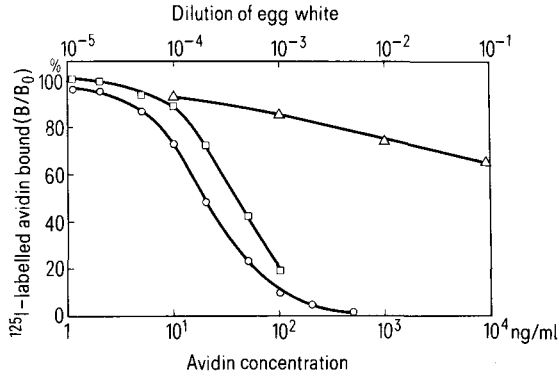
Summary. Biotin-binding was found in the egg whites and yolks of all 23 avian species studied, and in a turtle, but the amount varied considerably even in related species. There was no clear correlation in biotin-binding between egg white and yolk in various species. Antigenic determinants of avidin in different species have changed in the course of evolution as compared with those of chicken egg white avidin.

Avian egg white and the egg jelly of the frog contain a specific biotin-binding protein called avidin[2-6]. A biotin-binding protein distinct from avidin has recently been discovered in the chicken egg yolk[7,8]. In contrast to avidin, the yolk biotin-binding protein is saturated with biotin, and a special biotin-exchange assay is therefore required for its determination[7]. No comparative study of the occurrence of the egg white and yolk biotin-binding proteins has as yet been made. We therefore studied these proteins in the egg white and yolk in a number of avian and reptilian species, in fish hard roe, bull sperm and human seminal plasma.

Materials and methods. The eggs of 23 avian species, as shown in the table, were collected in southern Finland during the breeding period (April-July). 2 eggs of a turtle *(Testudo hermanni)* were utilized immediately after laying. The egg white and yolk samples were taken using separate syringes to avoid contamination, and diluted with the homogenization buffer used in the avidin assay[9]. The egg white and yolk samples, bull sperm and human seminal plasma were stored at −20 °C until assayed. The hard roe of whitefish *(Coregonus albula)* or perch *(Perca fluviatilis)* were assayed fresh.

Biotin-binding in the egg whites was assayed at room temperature as previously described[9]. Egg whites of 1 species in each family were also incubated at 100 °C to study the biotin saturation level and to show whether the protein has a heat stability similar to that of chicken avidin[10]. The biotin-exchange assay for egg yolks and hard roes was carried out at 65 °C as described by White et al.[7], and the ^{14}C-biotin-binding reaction in other samples at room temperature (21-22 °C). The avidin radioimmunoassay[9] was utilized to study in various species the presence of the antigenic determinants recognized by antiserum against chicken egg white avidin. The lipid material in the egg yolks was extracted with l-butanol[7].

Results and discussion. Biotin-binding was found in all egg whites and yolks studied (table) including *Larus argentatus*[5]. The biotin-binding activity varied considerably in the egg whites of different avian species even within the same family. In the major avian families, elevated temperature (100 °C) did not increase the ^{14}C-biotin-binding in the egg



Displacement of ^{125}I-avidin with chicken and quail egg white. Serial dilutions of the chicken (□) and quail (△) egg white were assayed by the radioimmunoassay for chicken avidin. Each point represents the mean of 4 determinations. Purified chicken avidin was used to obtain an avidin standard curve (○).

Biotin-binding activities in the egg white and yolk in various avian and reptilian species

Species[a]	Number of eggs	^{14}C-biotin bound (cpm × 10³/g)[b] Egg white	Egg yolk
Reptiles			
Chelonia			
Testudinidae			
Testudo hermanni	2	11.4	35.2
Birds			
Gaviiformes			
Gaviidae			
Gavia arctica	1	18	460
Anseriformes			
Anatidae			
Anas platyrhynchos[c]	9	33±4	59±2
Anas platyrhynchos[d]	7	180±29	84±4
Somateria mollissima	8	53±7	48±7
Melanitta fusca	5	218±29	209±4
Bucephala clangula	15	139±9	134±11
Galliformes			
Gallinae			
Gallus domesticus	10	290±13	88±2
Phasinidae			
Coturnix coturnix	9	513±64	57±7
Gruiformes			
Rallidae			
Fulica atra	4	372±44	130±15
Charadriiformes			
Haematopodidae			
Haematopus ostralegus	1	77	59
Stercorariidae			
Stercorarius parasiticus	1	165	-
Laridae			
Larus ridibundus	15	37±4	132±15
Larus argentatus	3	20±11	123±11
Larus canus	2	57	183
Sternidae			
Sterna hirundo	5	158±18	134±18
Columbiformes			
Columbidae			
Columba livia	2	57	183
Passeriformes			
Turdidae			
Phoenicurus phoenicurus	3	73±1	156±13
Turdus pilaris	4	26±3	97±9
Muscicapidae			
Muscicapa striata	3	9±0	136±7
Ficedula hypoleuca	6	46±7	48±4
Paridae			
Parus major	9	317±20	233±11
Corvidae			
Pica pica	2	22	143
Corvus corone	8	106±15	154±22

[a] Orders and families are also indicated. [b] The means ± SEM are given. [c] Domestic form. [d] Wild form.

white (not shown), which suggests that avidin is essentially biotin-free. On the other hand, heating decreased biotin-binding values in some avian species, suggesting a smaller stability of their avidin to heat than that in the chicken.

The quail egg white showed an incomplete cross-reaction in the radioimmunoassay for chicken avidin (fig.), while the egg whites of other avian species could not prevent ^{125}I-labelled avidin from binding to antiserum. This result indicates differences in the antigenic determinants of avidin molecules as compared to chicken avidin.

The biotin-binding protein found in the chicken egg yolk[7,11] was demonstrated here to be a common constituent in the egg yolk of various avian species (table). This protein is distinct from avidin, since it is denaturated at 100 °C[7,10]. The biotin-binding activities in the egg yolks also varied considerably from species to species. No clear correlation was found between the biotin-binding activities in the egg white and yolk in different species. The lipid-free yolk material in all avian species studied did not show any cross-reaction in the avidin radioimmunoassay.

The egg white and yolk of the turtle also showed biotin-binding activity (table), but no cross-reaction in the avidin radioimmunoassay. No biotin-binding activity was found in the hard roe of the fishes, bull sperm or human seminal plasma. Jones and Briggs[5] discovered a low biotin-binding activity in fresh bull sperm. This discrepancy in results might originate in the microbiological avidin assay they used, since the growth of microbes could be inhibited by any growth inhibitor present in the bull sperm.

It has been proposed that the biotin-binding proteins might be widely distributed in the animal kingdom and play some fundamental role in the physiology of reproduction[3-5]. An antimicrobial[6,12] effect for avidin, and a biotin-transporting role[11] for yolk biotin-binding protein, have been suggested as their functions. Fishes and mammals[13-16] so far studied did not contain any biotin-binding protein similar to that found in egg whites and yolks. It seems possible that special biotin-binding proteins have evolved for reproductive purposes in amphibian, reptilian and avian eggs.

1 We thank Mr Jukka Peltonen and Mr Antti Karlin for the collection of the avian eggs with permission obtained from the Ministry of Agriculture, and Mr Reino Saarinen for the turtle eggs. The authors are indebted to Mrs Outi Kurronen, Miss Riitta Mero and Miss Tiina-Maija Mattila for technical assistance. This work was supported by the Ford Foundation Grants No. 760-0526 and No. 790-0665.

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A low molecular weight tracer molecule for immunocytochemistry. Identification of cytoplasmic actin

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Summary. Anti actin Fab-fragments were tagged to a small electron dense tracer molecule; ferrocene monocarboxylic acid (230 daltons). The conjugate stains actin filaments, which were found mainly in the core of microvilli.

Many technical efforts have been made to visualize antigenic structures immunocytochemically. The main obstacle has been that the methods depend upon very large tracer molecules such as ferritin[1]. The immunoperoxidase method[2] does not eliminate this problem either, as the enzyme-antibody complex is still too large in diameter to pass through the cell membrane. Attempts were also made to allow large molecules to penetrate the plasma membrane with membrane disrupting agents[3,4], or with enzymatic digestion of certain membrane components[5]; however, these manipulations resulted in the destruction of the cell shape. Thus most techniques are still far from being established for immunocytochemistry. We here present a more suitable staining procedure, which helps to avoid most of the difficulties mentioned above. The very small Fab-fragment - ferrocene carboxylic acid (FMCA) complex identifies intracellular antigens without background effects. The procedure is easy to handle, direct and does not result in the destruction of cellular ultrastructure.

Actin was isolated from Ehrlich mouse ascites tumour (MAT) cells according to Lazarides and Weber[6], purified by polymerization- depolymerization cycles[7] and by DNase-I affinity chromatography[8]. Actin was injected s.c. into male New Zealand rabbits in the presence of complete Freund's adjuvant (protein concentration: 1.5 mg/ml). This was repeated on days 8 and 40 after the 1st inoculation. The IgG fraction was isolated from the serum and purified by DEAE-52 ion exchange chromatography[9]. Fab-fragments were prepared according to Porter[10] and labeled with the iron-containing sandwich molecule FMCA, using a water soluble carbodiimide[11]. Fab-fragments (5 mg protein), FMCA (5 mg) and 1-ethyl-3 (3-dimethylaminopropyl)-carbodiimide (10 mg) were dissolved in 2.5 ml 10 mM sodium phosphate and gently stirred at 4 °C over night. The