

mal and distant from the wound scar were ten small, well vascularized nodules of lymphoid tissue.

Small fragments of one liver tumour (Case 5) were washed several times in sterilized physiological solution and placed in the dorsal lymph sac of six immature *Xenopus laevis laevis*. The results from this experiment are summarized in Table II and clearly indicate that the benzpyrene-induced tumour was readily transplantable, advanced tumours being found in the kidneys, liver (Figure) and spleen.

From Table III it will be seen that, while Experiment II was less successful than Experiment I, 9 of the 20 treated animals bore lymphoid tumours of liver and of kidneys, spleen or abdominal wall muscle when they were killed after 272 to 310 days.

As with methylcholanthrene a considerable time was necessary between crystal implantation and the appearance of certain tumours. This long latent period may explain the failure of two previous experiments<sup>6,7</sup> using benzpyrene with anuran amphibians, in which all the treated animals died within a short time.

From the above experiments and others described elsewhere<sup>2</sup> it is clear that both benzpyrene and methyl-

cholanthrene lead to the development of transplantable lymphoid sarcomas when placed in the anuran amphibian, *Xenopus laevis laevis*<sup>8</sup>.

**Résumé.** Quelques cristaux de benzopyrène furent implantés dans trente-trois amphibiens anoures (*Xenopus laevis laevis*), vingt d'entre eux montrèrent des lymphosarcomes à différents endroits, le plus souvent localisés dans le foie et les reins. Ces tumeurs induites furent transplantées à nouveau avec succès<sup>1</sup>.

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<sup>6</sup> F. DURAN-REYNALS, Yale J. Biol. Med. 11, 613 (1939).

<sup>7</sup> J. SKAPIER, Acta Un. int. Cancr. 6, 65 (1948-50).

<sup>8</sup> This work was supported by the Fonds national suisse pour la recherche scientifique (No. 2219). The author wishes to thank Profs. M. FISCHBERG and A. W. BLACKLER for their advice.

### Immunoelectrophoresis of Avian Lens Proteins

While in a previous paper the relationships of *Afropavo* within the Galliformes were studied, both by electrophoretic and immunoelectrophoretic methods<sup>1</sup>, the present work deals with a condensed general review of some different immunoelectrophoretic patterns, obtained from the representatives of some phylogenetically important birds.

The lenses were homogenized in *aqua dest.* (200 mg fresh tissue per ml). After centrifugation (20,000 × g for 20 min) the clear supernatant was used for electrophoretic and immunoelectrophoretic examination. Electrophoresis was carried out as described earlier<sup>2</sup> and the relative mobility of the fractions was determined using the known mobility of a test substance<sup>3</sup>.

We used antiserum against the soluble proteins of starling lenses (*Sturnus vulgaris*). Combined electrophoresis and immunoelectrophoresis were performed according to RABAEY<sup>4</sup> on the same plate, which enables exact correlation of the two patterns. All plates are reproduced on the same scale, thus being directly comparable.

The lens antigens of the birds examined were tested with anti-starling antiserum, and thus it may be evident that all immunological patterns can only reflect relationships with the Passeriformes. Some species, however, reacting with the anti-starling antibodies, show a resembling or even similar pattern among each other. Consequently some conclusions about the mutual relationships can be drawn.

Among the several patterns discerned, the one in which most antigens give an individual precipitation line is the most widespread. To this type belong all orders in which the typical song bird component (rel. mob. 0.60 or 0.50) occurs<sup>2</sup>, namely the Ciconiiformes, Falconiformes (*Accipiter nisus*, Figure 1), Strigiformes, Gruiformes, Psittaciformes, Coraciiformes, Piciformes and Passeriformes. It is also the type of the Charadriiformes, Procellariiformes

and Columbiformes, and finally also of the Galliformes, Rheiformes and Casuariiformes.

In a second type, the precipitation lines of several antigens form one single stretched and sharp-cut line. This is the type of the Anseriformes, Podicipediformes and Gaviiformes, and also of *Alca torda* and *Cochlearius cochlearius*, which is represented in Figure 2.

Thirdly, the rather simple electrophoretic runs of the Sphenisciformes, the Phalacrocoracidae-Sulidae (*Sula bassana*, Figure 3) and of *Uria aalge* show a simple but characteristic immunological reaction.

Among the birds with rather aberrant patterns, we would cite *Cuculus canorus*, where in spite of an apparently reduced electrophoretic pattern, the immunological reaction shows more well developed and sometimes sharp-cut precipitation lines.

A discussion of all patterns and their similarities in detail would lead us too far, but meanwhile it is clear that the immunological results confirm a lot of conclusions already drawn from the electrophoretic runs.

At first the resemblances between all Passeriform lens pherograms (showing the typical 0.60 fraction) are accentuated by their analogous precipitation patterns in immunoelectrophoresis.

Secondly, some presumed relationships between the birds with an Anseriform pattern could be confirmed (e.g. grebes-loons-auks), while also new perspectives are opened (ducks-*Cochlearius*-herons).

Finally there is no doubt that all birds with a Sphenisciform pattern are pretty closely related. The rather astonishing position of *Uria aalge* is confirmed by the absence

<sup>1</sup> H. GYSELS and M. RABAEY, Bull. Soc. zool. Anvers 26, 72 (1963).

<sup>2</sup> H. GYSELS, Exper. 19, 107 (1963).

<sup>3</sup> M. RABAEY and G. VERRIEST, Ann. Soc. zool. Belg. 88, 373 (1958).

<sup>4</sup> M. RABAEY, Exp. Eye Res. 1, 310 (1963).

The immunological reactions indicated with an apostrophe refer to the corresponding numbers of the electrophoretic runs. Besides each bird examined, the immunoelectropherogram of *Sturnus vulgaris* (starling) was represented (s) to allow a comparison with the most complete immunological reaction. d = dextran (rel. mob. = 0); a = human serum albumin (rel. mob. = 1); ag = antiserum groove; ig = insertion groove;  $\alpha$  =  $\alpha$ -crystallin.

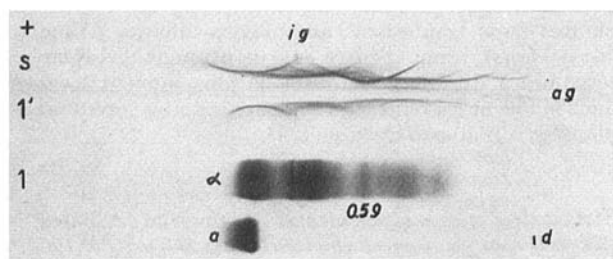


Fig. 1. In a Passeriform lens pherogram, characterized by the 0.60 fraction which is found, for instance, in *Accipiter nisus* (sparrow hawk), almost all components give an individual precipitation line

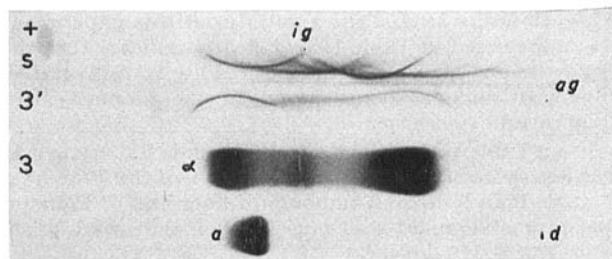


Fig. 3. Immunoelectropherogram of *Sula bassana* (gannet). Besides the one of the  $\alpha$ -crystallin, the simple reaction shows a characteristically curved precipitation line.

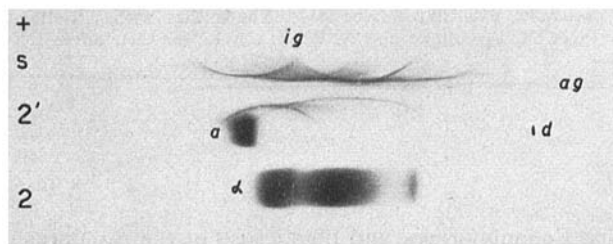


Fig. 2. Immunoelectropherogram of *Cochlearius cochlearius* (boat-bill heron) showing the Anseriform type: a long extended fused precipitation line corresponding probably, as in ducks, to the multiple antigens of the FISC (RABAEY<sup>4</sup>).

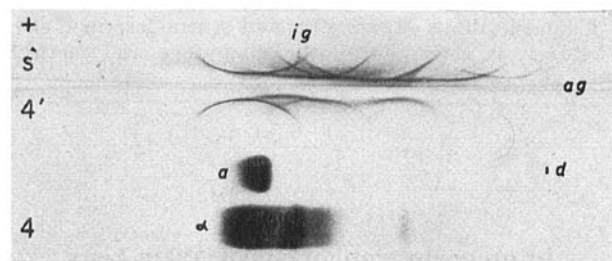


Fig. 4. Among other types, *Cuculus canorus* (European cuckoo) represents an apparent reduced lens pherogram, the less visible components of which, however, are revealed by a more complete immunological reaction.

of glycogen in its lens<sup>5</sup>. While all Charadriiform lenses contain glycogen, it is not found with Sphenisciformes and 'Pelecaniformes' (i.e. gannets and cormorants, for *Pelecanus* has a glycogen-positive, heron-like lens pherogram, and perhaps should rather fit in the Ciconiiformes; cf. the old conception of GADOW, 1893<sup>6</sup>). More detailed reports upon this matter will soon be published elsewhere<sup>7</sup>.

**Zusammenfassung.** Mit Immunelektrophorese und Mikro-Agarelektrophorese werden die Linsenproteine verschiedener Vogelgruppen untersucht. Der Vergleich der Reaktion der Linsenextrakte mit anti-Star-Antiserum erlaubt nicht nur Rückschlüsse auf die Verwandtschaft mit den Singvögeln; auf Grund ihrer ähnlichen Reaktion

kann auch die gegenseitige Verwandtschaft der untersuchten Arten beurteilt werden.

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<sup>5</sup> M. RABAEY, *Nature* 198, 206 (1963).

<sup>6</sup> H. GADOW, in BRONN's *Klassen und Ordnungen des Tierreichs*, Vögel II (1893).

<sup>7</sup> **Acknowledgments.** I wish to thank Professors Dr. L. DE CONINCK, J. M. DENUCE and M. RABAEY for their most critical survey of this work, both on the taxonomic and the biochemical plan.

### Azione dell'«Epidermal Growth Factor» sulla sintesi di acidi nucleici e proteine dell'epitelio cutaneo<sup>1</sup>

È di recente acquisizione il fatto che la crescita e la differenziazione di alcuni tipi cellulari è direttamente influenzata e regolata da specifici «growth factors». Il primo di questi fattori ad essere individuato e caratterizzato nella sua struttura, agisce selettivamente sulle cellule nervose simpatiche ed è riportato nella letteratura come

NGF (LEVI-MONTALCINI e COHEN<sup>2</sup>; LEVI-MONTALCINI e ANGELETTI<sup>3</sup>). Più recentemente è stato isolato un altro fattore; che agisce stimolando specificamente la crescita

<sup>1</sup> Il presente lavoro è stato realizzato con fondi del NIH e della Merck Sharp-Dohme Co.

<sup>2</sup> R. LEVI-MONTALCINI e S. COHEN, *Ann. N.Y. Acad. Sci.* 85, 324 (1960).

<sup>3</sup> R. LEVI-MONTALCINI e P. U. ANGELETTI, *Quart. Rev. Biol.* 36, 99 (1961).