New Biochemical Techniques Applied to Avian Systematics

The technique of electrophoresis was used more than once to obtain taxonomical data about birds: thus egg-white[1,2], plasma proteins[3,4], hemoglobins[5,6] and lens proteins[7] were examined. The best fractionation can be obtained by using agar medium, and reliable taxonomical criteria can also be realized by accurate measurement of mobility. To prepare the electrophoresis slides, a 1–2 mm layer of Difco Bacto agar was allowed to gel on a glass slide of 9×4 cm. Electrophoresis was carried out at a constant temperature of 4°C (cooled by petroleum ether) in veronal-buffer pH 8.4, ionic strength 0.03 with a potential of 20 V/cm for 25 min. The extracts were made by homogenizing the organs with the addition of a fivefold quantity of distilled water for the lens, and about a double quantity for the muscles. After centrifugation at 15000 *R*/min (7 min) a drople of the clear centrifugate was brought into the agar. The mobilities were calculated by using the known mobility of a test substance[8].

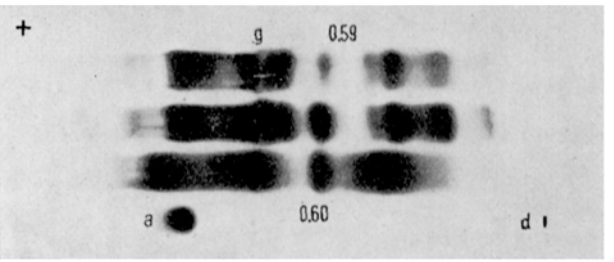
The soluble proteins of lens and muscles gave good results, both for the number of components obtained, and for the clear separation. As already demonstrated by paper electrophoresis, protein composition of birds' lenses shows a great biochemical differentiation[7]. No general pattern exists; only one fraction seems to correspond with what in mammals is called α-crystallin. Within the same species, pherograms always have the same aspect and they are also quantitatively rather constant. Some fractions, however, lose their sharp-cut character with increasing age. Of course this should be taken into account for comparative experiments, but a real variability does not exist.

In the order of Passeriformes, a sharp-limited fraction is regularly seen, the nature of which has not been determined yet, and which has, as far as we know, never been mentioned by any other investigator. The mobility may vary slightly between the families, but always has an average value of 0.60. An example of such a slight but significant variation can be seen in the Hirundinidae, where the fraction clearly runs slower than in most other Passeriformes: 0.59 (Figure 1). It is quite remarkable that this fraction also occurs in the lens pherograms of the Strigiformes, Gruiformes, Ciconiiformes, Psittaciformes, Coraciiformes, and the Buteonidae, but in a smaller concentration. In the Falconidae and the Piciformes there is no 0.60 fraction, but there is one with mobility 0.50 (Figure 2). Other orders show quite different lens pherograms: in the Anseriformes, there are always about five clear fractions with approximately equal distances between them, in the Charadriiformes there are two clear fractions, which however quickly lose their sharp-cut character with increasing age. Columbiformes have a lens pherogram which is different from all other orders (Figure 3).

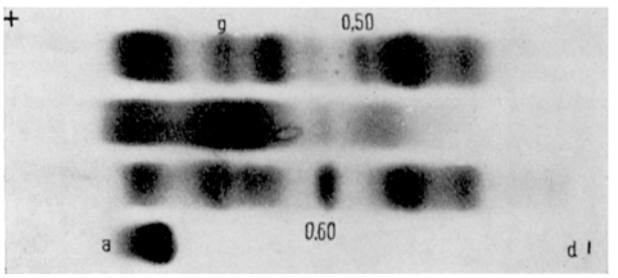
Muscle pherograms show a similar pattern for all birds, in the sense that there is always one main fraction in strong concentration, probably myogen or part of it (HAMOIR, personal communication), and more to many more less clear fractions with very different mobilities. The mobility of the main fraction has also an important taxonomic value, while it is often characteristic for the genus and even the family. In all examined Passeriform families, it has an average value of 0.21, except for the Corvidae, Oriolidae, Sylviidae, Regulidae and Timaliidae, where it has a value of 0.32 (Figure 4). The same fact holds within other orders. In the Buteonidae the mobility of the main fraction amounts to about 0.26, in the Falconidae it is about 0.32. Within the family Psittacidae, we find for the parrots 0.23, and for most parakeets 0.31. In the order of the Galliformes, the Numididae show a main fraction with mobility 0.34, the Phasianidae however show 0.41.

Protein fractions with enzymatic activity may also have a systematic value. A staining method with benzidin was adapted to characterize the myoglobin with per-

Hirundo rustica, Swallow; *Anthus trivialis*, Tree Pipit; *Anthus pratensis*, Meadow Pipit



Falco tinnunculus, Kestrel; *Buteo buteo*, Buzzard; *Alauda arvensis*, Sky Lark



Anas platyrhynchos, Mallard; *Vanellus vanellus*, Lapwing; *Actitis hypoleucos*, Common Sandpiper; *Columba livia*, Pigeon

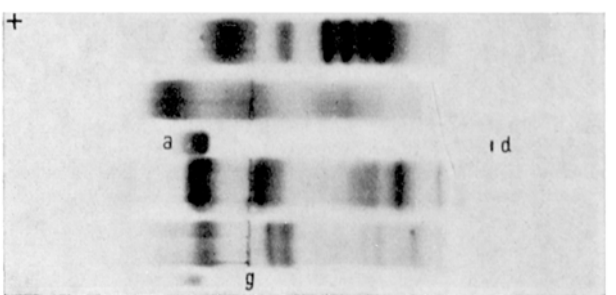


Fig. 1, 2, 3. Lens pherograms. Human serum albumin (a) is used as a reference, whereas zero migration is indicated by dextran (d). The insertion groove (g) and the 0.60, 0.59 and 0.50 mobilities are also represented.

[1] R. A. MCCABE and H. F. DEUTSCH, Auk *69*, 1 (1952).
[2] C. G. SIBLEY, Auk *102*, 215 (1960).
[3] R. H. COMMON, W. P. MCKINLEY, and W. A. MAW, Science *118*, 86 (1953).
[4] H. F. DEUTSCH and M. B. GOODLOE, J. biol. Chem. *181*, 1 (1945).
[5] J. S. DUNLAP, V. L. JOHNSON, and D. S. FARNER, Exper. *12*, 352 (1956).
[6] A. SAHA, R. DUTTA, and J. GHOSH, Science *125*, 447 (1957).
[7] M. RABAEY, Aggr. thesis Rijksuniv. Gent, Belgium (1959).
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Pica pica, Magpie; *Garrulus glandarius*, Jay; *Chloropsis aurifrons*, Goldfronted Leafbird

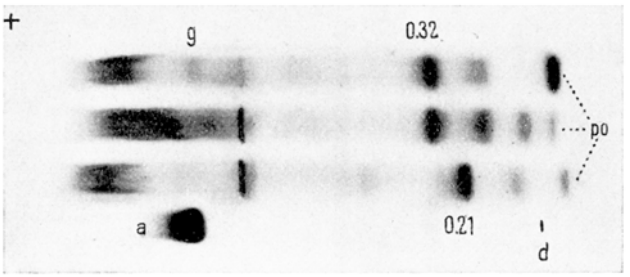

Fig. 4. Muscle pherograms showing the 0.32 and 0.21 mobilities of the myogen and also the myoglobin with peroxydase activity (po).

Turdus merula, Blackbird; *Turdus pilaris*, Fieldfare; *Turdus philomelos*, Song Thrush; *Turdus iliacus*, Redwing

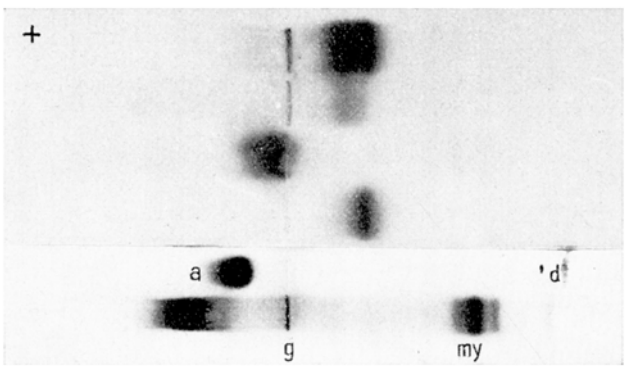

Fig. 5. Muscle pherograms with specific staining for esterases. The lower part of the Figure illustrates a control-strip with serum albumin (a) as a test. It was stained in the usual way with amido-black, showing also the mobility of the myogen of the Redwing.

oxydase activity. Esterase iso-enzymes and LDH are quite easily detectable in a bird's lens (RABAEY, personal communication) and suitable for mobility measuring. Esterase was detected by using α-naphthyl-acetate as a substrate; LDH by a histochemical method[9], while the substrate was brought in according to an enzymo-electrophoretic method (WIEME, personal communication). Also in the muscle extract of birds, those enzymes are detectable and the esterases are sometimes very specific. This is shown clearly by four *Turdus* species (Figure 5).

Research is being continued on birds of families and orders not yet examined, and also on the comparison of lens proteins by immuno-electrophoretical methods[10].

Résumé. Les protéines solubles de la lentille et des muscles des oiseaux furent examinés au moyen de la micro-électrophorèse en agargel. La mobilité des fractions protéiques s'est présentée comme un caractère systématique important: il y a généralement des différences nettes et constantes, mais parfois aussi des ressemblances, qui indiquent probablement des relations de parenté.

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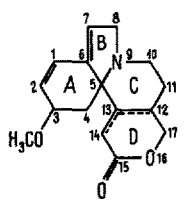
[10] *Acknowledgments.* I am much indebted to the late Prof. Dr. R. VERHEYEN, and to Prof. Dr. M. RABAEY, whose knowledge and councils were of extreme value for me. I wish to thank also Mr. W. VAN DEN BERGH, director of the Antwerp Zoo, and the co-workers of the Belgian Ringing Work, who provided me with so many birds.

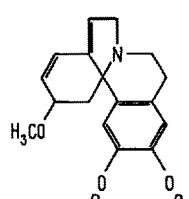
STUDIORUM PROGRESSUS

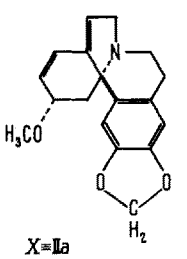
Absolute Configuration of the Spiro Carbon Atom of the Erythrina Alkaloids: Evidence from Optical Rotatory Dispersion

Plants of the genus Erythrina (Leguminosae) produce two groups of dienic alkaloids: the lactones α- and β-erythroidine (Ia and b, resp.), and a number of aromatic bases of the general formula II[1].

While the structures of the bases have been unequivocally established, the stereochemistry is only incompletely known. For the lactone base Ia, HILL and SCHAERER[2] have established the absolute configuration at C-12 which is asymmetric in this one alkaloid only; this configuration, however, has not been correlated with those of the two other asymmetric centers, C-3 and C-5, which are present in all Erythrina alkaloids[3]. Since Ia and Ib have been interrelated[4], their configurations at C-3 and C-5







[1] For reviews on these alkaloids, see (a) L. MARION, in *The Alkaloids* (Ed. H. L. HOLMES and R. H. F. MANSKE, Academic Press, Inc., New York 1952), vol. II, p. 499. – (b) V. BOEKELHEIDE, in *The Alkaloids* (Ed. H. L. HOLMES and R. H. F. MANSKE, Academic Press, Inc., New York 1960), vol. VII, p. 201.

[2] R. K. HILL and W. R. SCHAERER, J. org. Chem. *27*, 921 (1962).

[3] Unpublished evidence on the absolute configuration of C-3 in Ia and Ib is mentioned in [2].

[4] V. BOEKELHEIDE and G. C. MORRISON, J. Amer. chem. Soc. *80*, 3905 (1958).