Color Change Mechanisms of Cold-Blooded Vertebrates

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Foreword

An outstanding figure in the emergence of experimental biology immediately after World War I was Lancelot Hogben. That generation of zoologists and physiologists know well how much they owe to his brilliant leadership in what was then a new and exciting field, and to his early book on comparative physiology.

Of his varied experimental interests one of the most important was that of color change in the vertebrates and its relation to endocrine control. The elegance and interest of his development of this part of vertebrate comparative physiology was evident at once. But the fundamental light which it could throw on basic physiology and on physiological adaptation was not perhaps generally appreciated till after the realization during the 1930's of the profound importance of the chemical transmission of excitation and the endocrine control of biochemical processes.

It is therefore with particular pleasure that we see here a very clear and careful assessment of the present position of the vertebrate color-change problem, and to what new goals research should be directed, by one of Dr. Hogben's outstanding research collaborators in this field. Those of us who, like Professor Waring, stand in great debt to the early intellectual stimulus of Lancelot Hogben and his work will particularly welcome Professor Waring's thorough and far-sighted review.

C. F. A. PANTIN

University of Cambridge
England
April 1963

Acknowledgments

Many colleagues, former and present, have helped with criticisms of my drafts in their specialist fields. In particular I wish to mention in this connection—Drs. Bartholomew (California), Dawson (Michigan), Healey (London), Horowitz (Chicago), Ketterer and Main (West Australia), Landgrebe (Cardiff), and MacFarlane and Woolley (Canberra).

Anyone familiar with the administrative and teaching demands on the head of a service department in an Australian University will appreciate that I wish to acknowledge generous relief and support. My University has recently permitted me 12 months' freedom from routine duties and Fulbright and Carnegie made travel and laboratory work abroad possible. It was during the leisure moments of those 12 months that this monograph was written. Ernest Hodgkin, by taking extra burdens, made my leaves possible. My wife has provided strong moral support. It is a pleasure to acknowledge skilled and rapid typing by Mrs. Crapp who also undertook the tedious task of cross checking. Dr. Zwicky of this University kindly read the whole manuscript.

This monograph has been in mind for several years. Its appearance has been delayed to coincide with the retirement of Lancelot Hogben—as a tribute to him. Brilliant, mercurial, and unquestionably difficult at times to older men, he has given to me and many of my generation all a young man should ask from a professor—stimulus, intellectual substrate, facilities, unstinted help without the now common predation of putting his name on my papers, loyalty, and affectionate guidance in many things.

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H. WARING

Contents

CHAPTER 1

Introduction

Animal color change was known to the ancients, and Aristotle described with remarkable accuracy the changing tints of devil fishes and chameleons. Although color change mechanisms have been studied in many laboratories during this century, most noteworthy advances can be traced to schools centered on, and deriving ideas from, three men—von Frisch (Germany), Hogben (United Kingdom) and Parker (United States). Parker's "Animal Colour Changes and Their Neurohumors" published in 1948, will remain a standard source work for many years, but there is internal evidence in it (reinforced by correspondence) that Parker did not appreciate fully the significance of much work from the Hogben group; this state of affairs is of course commonplace in any scientific discipline. There is therefore a need for another statement of information and interpretation, and this monograph is an endeavor to present a connected account of the work of Hogben's school, with of course cognate work by others taken into account.

The volume is addressed directly to senior undergraduate students in zoology, physiology, pharmacology, and biochemistry, but it could well be useful as ancillary reading for some students proceeding to the Ph.D. The emphasis I have placed on method and inference leads me to hope that it may be of use also to students of the humanities reading scientific method in Philosophy, and animal behavior in Psychology.

There is information about color change from invertebrates, cold-blooded vertebrates, birds, and mammals; and most research groups have restricted themselves to one, or two, of these. A book on the whole subject would consequently involve an author in synthesis at the purely verbal level in many areas. This monograph attempts to present a connected account of mechanism in cold-blooded vertebrates alone. A few key references to work on invertebrates, birds, and mammals is appended for the convenience of students.

Chapter 2 considers briefly the kinds of color cells, the classes of response

and their measurement, and the terminology of hormones involved in color change. Chapters 3.1 to 3.5 deal systematically with each vertebrate class. Each chapter opens with an account of one or more examples that are best documented, and this, where feasible, is followed by attempts at generalization involving information from animals about which there is less complete information. Chapters 4 to 6 describe the biological assay, chemistry, and pharmacology of pituitary hormones involved in color response. Chapter 7 considers morphological color change, Chapter 8 the possible survival value of color change, and Chapter 9 the possible implication of the pituitary hormones responsible for color change in other physiological processes.

CHAPTER 2

Resume of Responses

Origin and Nomenclature of Chromatophores

Chromatophores of vertebrates originate in the neural crest (Du Shane, 1943; Horstadius, 1950; Willier, 1953) and migrate to their final destination which may be the peritoneum, dermis, or epidermis. It is customary to refer to color cells which contain red, yellow, and brownish-black pigment granules as erythrophores, xanthophores, and melanophores, respectively; the chemistry of the pigments has been described by Fox (1953). Erythrophores, and xanthophores, will be referred to only incidentally in this monograph. In recent years there has been some confusion due to the growing use of the term melanocyte. The International Conference on Pigment Cell Growth (1953) recommended uniform usage of melanocyte, melanophore, etc., and Gordon (1959) followed this up with evidence that melanoblasts, melanocytes, and melanophores are successive stages of the same cell in ontogeny. Here we will be concerned only with the definitive cell of poikilotherms, the pigmentary effector or melanophore.

Physiological and Morphological Color Changes and Their Measurement

Two kinds of pigmentary response can be easily distinguished, the so-called morphological, and physiological. Morphological changes are those, shared with mammals, in which there is an absolute build-up of pigment and melanophores. Physiological changes, not shown by mammals, involve redistribution only of pigment within the melanophore. Figures 2.1, 2.2, 2.3 and 2.4 show examples of the macroscopic effect of physiological change and the low power microscopic appearance of skin in pale and dark phases. Although melanoblasts or melanocytes (as defined above by Gordon) may exhibit amoeboid movement, all evidence is consistent with the melanophore

having a "static" boundary and pigment moving within it in response to stimuli. Consequently, the old terms "expansion" and "contraction" of melanophores are not used. The terms "dispersion" and "aggregation" of pigment seem to be now generally acceptable and are used here.

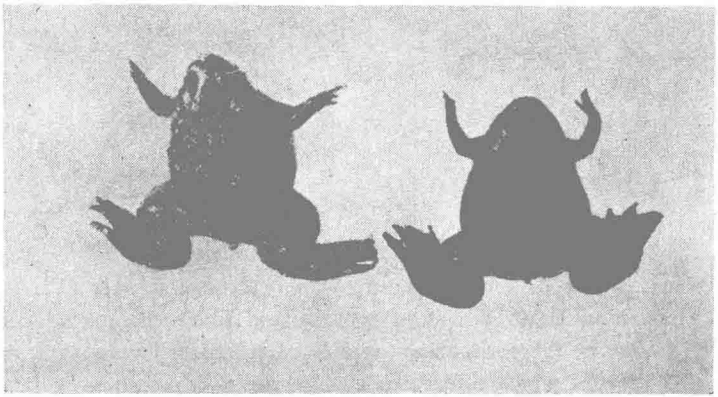

FIG. 2.1. *Xenopus*, the South African clawed toad in the dark and pale phase. (From Hogben and Slome, 1931.)

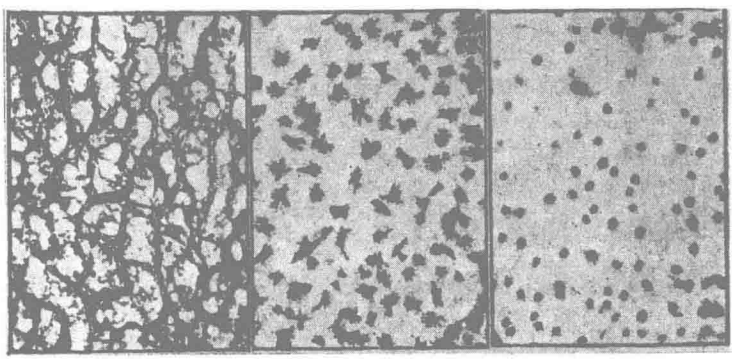

FIG. 2.2. The appearance of amphibian melanophores under low power microscope in dark, intermediate, and pale phases. (From Hogben, 1924.)

Both morphological and physiological changes need accurate assessment for quantitative work. The conditions resulting in melanin dispersion, or its reverse, also cause, respectively, absolute increase or decrease of melanin, so that in fact the two processes overlap in time. But morphological change is so slow compared with physiological change that no significant confusion can arise between them if the melanophore index (m.i.; see below) is used.

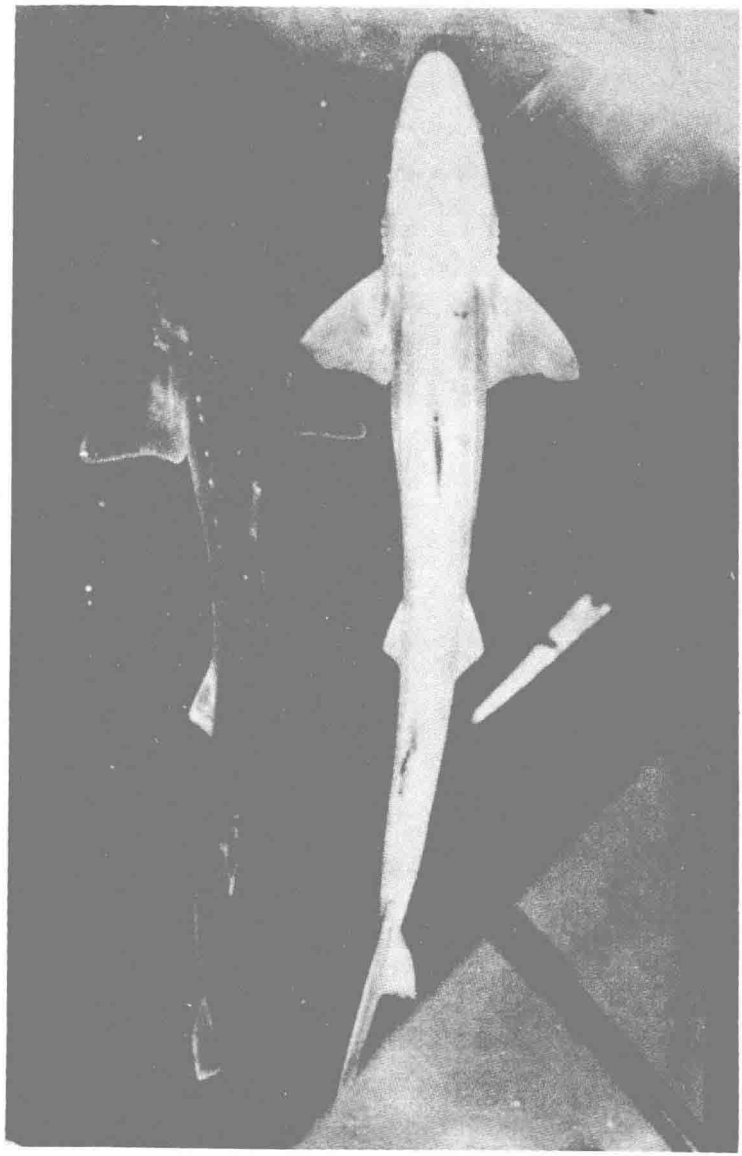

FIG. 2.3. *Squalus* (dogfish) in the dark and pale phase. (From Waring, 1938.)

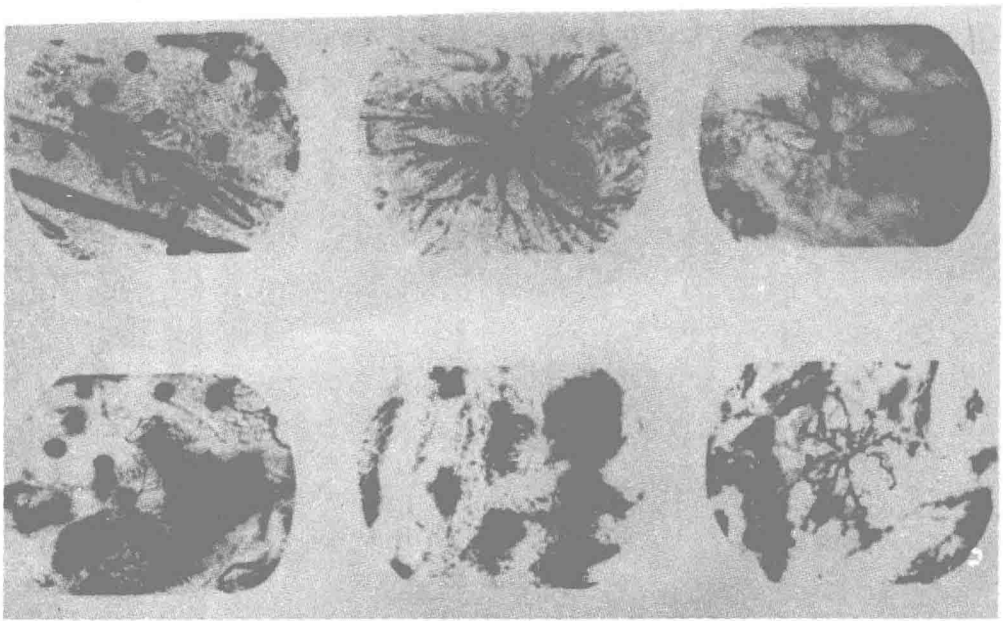

FIG. 2.4 *Squalus* (dogfish) melanophores at various phases. A. Epidermal and dermal melanophores; melanin fully aggregated. B. Dermal melanophores; melanin partially dispersed. C. Dermal melanophores; melanin fully dispersed. D. Epidermal melanophores; melanin partially dispersed. E. Epidermal melanophores; melanin fully dispersed. F. Static epidermal pigment. (From Waring, 1938.)

Since measurable morphological change takes days or weeks, counting the number of melanophores per unit area and extracting melanin and measuring it photometrically is perfectly adequate (see Chapter 7).

Physiological changes are more rapid, sometimes with significant changes within 3 minutes. Early workers were content to describe this kind of behavior in terms of macroscopic appearance of the animal as dark, intermediate, pale, etc. For purposes of natural history vis-à-vis the protective coloration controversy, a case can be made for recording the gross macroscopic *as well as* the individual behavior of the melanophores, but for physiological studies bearing on the behavior of an individual effector organ and its coordination there are several valid objections to the system. The terms used are inexact. Hence, the records of different workers are not comparable, and communications are verbose. A more important objection, which also applies to photoelectric recordings of large areas of skin, is that macroscopic appearance depends on the sum total of chromatophores and on the previous history of the animal. The last point is of particular importance. Animals kept under conditions that maintain dispersed melanin in the melanophores develop more melanophores and more melanin. Under conditions that evoke melanin aggregation the reverse happens. This is emphasized in Fig. 2.5, showing that it is possible for an animal with many melanophores to be darker when all its melanin is aggregated, than another animal with fewer melanophores but with the pigment dispersed. By the use of the Hogben melanophore index (Fig. 2.6) these pitfalls are avoided, and a precise estimation of individual effector activity is obtained. Objections have been raised to the use of the index on two grounds: (*a*) that the subjective element in assessment is large, and (*b*) mistaken interpretations have arisen through failure to realize that the assigned numerals are arbitrary. With regard to (*a*), experience with two workers reading on each occasion, and years of class work, leave no doubt that disparity between individual readers is small, but to reassure people inexperienced in this field a test was performed, detailed in Chapter 4, which showed that the error from this source is less than 10%. More recently, Thing (1952) made a direct comparison between photoelectric determinations and visual assessment of the melanophore index (m.i.) during transitions on one animal, where the above mentioned criticism of the former methods does not apply. He concluded that the m.i. readings were at least as reliable as photoelectric methods, and more convenient. With regard to (*b*), while it is impossible to be certain that nobody has been misled by this, I am not aware of any recent worker who thought the figures were other than arbitrary. Roggen (1962) "aimed at giving the Hogben-Slome index a more objective, hence more reliable aspect by determining its

quantitative value." With a planimeter he measured the surface area of melanin at different phases of melanin dispersion. His measurements permit his conclusion that there is no statistically significant difference between 1 and 2 on Hogben's scale. He showed that if 1 and 2 on the Hogben scale are merged to become 1 on a new four-point scale, and that 4 on the new

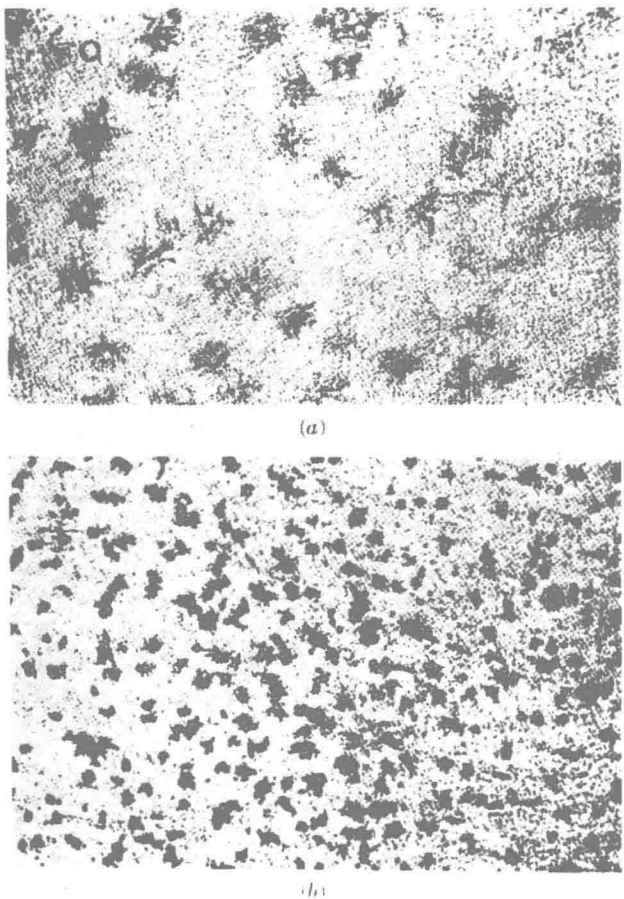

Fig. 2.5. To show how a combination of reduced melanophores per unit area and melanin per melanophore may result in a skin with melanophores equilibrated at melanophore index (m.i.) = 2, being macroscopically darker than skin at m.i. 5. Magnification of both photographs the same; we have *Xenopus* with less melanin than in (a) but these do not photograph well with the melanin dispersed. (From Landgrebe and Waring, 1944.)

scale is taken to indicate 100% dispersion, then 1, 2, and 3 will be very close to 25, 50, and 75%, respectively. Presumably, we are being invited to substitute the four-point scale for Hogben's five-point scale. Before doing so, the apparent conflict between Roggen's planimetric measurement of melanin dispersion and Main's (Chapter 4) assessment of it photometrically will need to be resolved. This aside, many people will see merit in retaining 1 and 2 in Hogben's scale because they are distinguishable visually under the microscope and, hence, are useful in describing the process of the change.

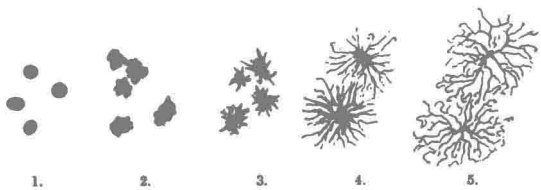

FIG. 2.6. Melanophore index. (From Hogben and Slome, 1931.)

The observation of the melanophore index in slow changing amphibians, with which gentle handling has little effect, is made simply by placing the web on the stage of a microscope. Observation of the melanophore index of most teleosts is difficult; the natural change is fast, and handling the fish interferes with the direction and speed of the change. Aside from the photoelectric method already mentioned, there have been two attempts to solve the problem. Wykes (1937) measured *fixed* melanophores. Observation on fixed material is unsatisfactory because even with slowly reacting melanophores, which are not appreciably affected by handling, fixation causes aggregation of fully dispersed melanin from 5 to about 4 on Hogben's scale. Similarly, fully aggregated melanin (m.i. = 1) is slightly dispersed by the action of even a quick-acting fixative such as Bouin. This degree of introduced error is important in connection with interpretations of the kind described in Chapter 3.1. The difficulty can be got around by either the method of Hogben and Landgrebe (1940) or that of Neill (1940) (Chapter 3.3), in both of which by use of sufficient animals, and a device for rapid reading, the index on any one animal is read only once so that any ill effects subsequent to, and caused by, the reading are not shown on the graph.

Classification of Responses

Melanophores respond to light in a variety of ways. For responses not involving the eye, the term nonvisual response will be used. Nonvisual responses are of two kinds: (*a*) coordinated by nerves or hormones, or (*b*)

TABLE 2.1

UNCOORDINATED AND COORDINATED NONVISUAL MELANOPHORE RESPONSES[a]

Animal	Independent effector	Evidence	Authority	Coordinated nonvisual response	Evidence	Authority
Ammocoetes of *Lampetra*	—	Skin of hypophysectomized animals is permanently pale under all circumstances	Young (1935)	+	After removal of pineal complex animals remain dark under all conditions of illumination	Young (1935)
Elasmobranch spp.	?	Blinded hypophysectomized *Mustelus* darkens in light, pales in darkness	Parker (1937)	+	Eyeless but otherwise intact *Raia* equilibrate at 3.0–3.5 according to lighting conditions	Hogben (1936a)
		Blinded hypophysectomized *Mustelus* show no response to light and darkness	Abramowitz (1939)		Eyeless *Mustelus* darken in light and pale in darkness	Parker (1937); Abramowitz (1939)
Ameiurus	—	Denervated skin remains permanently dark under all conditions of illumination	Wykes (1938)	+	Blinded animals with nervous system intact pale in darkness: darken in light	Wykes (1938)
Xenopus	+	Response evoked after destruction of central nervous system and removal of pituitary gland. Magnitude: about 0.5 on the melanophore index scale	Hogben and Slome (1931)	Probably +	Response of eyeless but otherwise intact animal operated on at least 5 years previously. Magnitude: animals equilibrate at 2.5 in darkness and at 5.0 in light	Landgrebe (unpublished)

[a] From Waring, 1942.