In a preparative experiment [mg scale] reactions were carried out in the following manner. The first amino acid was dissolved in 0.01 *N* hydrochloric acid, and the solution was added to the activated ion exchange resin. The mixture was heated for 10 min. The excess amino acid was removed by filtration after cooling the mixture and the resin was washed with cold water until ninhydrin positive material was removed. The resin was then put into a Meyer flask or packed in a glass column with a heating jacket. The second amino acid was dissolved in slightly acidic (hydrogen chloride) ethanol or methanol, and mixed with the resin in the Meyer flask or passed through the heated column slowly. The temperature of reaction, both by the column and the batch method, was about 60° (± 5°). Heating in the flask was made for about 15 h. Yields of the product were better in the column method than in the batch method.

Zusammenfassung. Eine neue Methode der Peptidsynthese, z.B. von Carnosin, GlySer, GlyGly, LeuTyr und GlyPhe mit Ionenaustauschharzen als Katalysatoren wird beschrieben.

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Table II. Comparison of Rf values and colour spots (by ninhydrin) of the reactants and the products on paper chromatograms and yield of the products determined spectrophotometrically

Reactant and product	Rf[a]	Colour of spot[b]	Yield of the product
β-Alanine	0.05	Indigo	
Histidine HCl	0.10	Violet	
Histidine Me ester	0.18	Violet	
Carnosine Et ester	0.23	Sky blue	[c]
Carnosine	0.35	Sky blue	
Carnosine Me ester	0.37	Sky blue	[c]
Glycine	0.17	Violet	
Serine	0.21	Violet	
Leucine	0.60	Violet	
Tyrosine	0.44	Violet	
Phenylalanine	0.55	Violet	
Glycylglycine	0.23	Violet	70%[d]
Glycylserine	0.20	Violet	25%
Leucyltyrosine	0.29	Violet	30%
Glycylphenylalanine	0.54	Violet	50%

[a] AcOH:*n* BuOH:H_2O = 1:4:5, upper layer. [b] By ninhydrin. [c] Yield was obtained spectrophotometrically at 467 mμ and listed in Table I. [d] Yield was obtained spectrophometrically at 570 mμ in the column method.

A Simple Method for Estimating Melanophore Responses to Drugs in Fishes

In a study of the reactions of fish melanophores to autonomic drugs, the need arose for a speedy, simple method for estimating the melanophore responses without handling or disturbing the fish unnecessarily. In the majority of teleost fish studied to date, the movements of pigment within the skin melanophores are coordinated by the sympathetic nervous system. In addition blood-borne pituitary hormones affect the state of melanophore pigment in similar, but slower fashion. These changes are conventionally referred to as 'physiological' changes, to distinguish them from the much slower 'morphological' changes in numbers of melanophores in the skin which follow prolonged exposure to one shade of background[1,2].

Recordings of the amount of melanophore response have been made in the past by observing the melanophores microscopically[3], or by observing the overall shade of the fish[4]. However, in the fish under study, *Phoxinus phoxinus* (L), handling the fish for microscopical examination brings about fluctuations of melanophore state[5]. The pharmacological study was carried out using the Derived Ostwald Index (DOI) of HEALEY[6] to discover whether standard grey papers could be used to estimate melanophore responses accurately. It was, however, necessary to discover the effects of morphological changes and of temperature on the observed shade of the fish.

Minnows were collected from Hertfordshire and kept in white or black stock tanks in the laboratory as described by HEALEY and Ross[6]. Fish from a stock tank were placed in beakers of tap water on trays painted the same shade as the stock tank and illuminated from above. The shades of the fish were estimated using the shade standards, and the beakers transferred to the opposite (black or white) background. Estimates of the shade of the fish were then made at intervals during which time the tap water was slowly changed to prevent fouling. Figures 1–4 represent the shade changes of the fish subjected to background reversal at different temperatures and after different histories on a particular background. Increase in DOI value indicates darkening, and vice versa, following the numerical convention adopted by HOGBEN[3] for his Melanophore Index (MI) based on microscopical observation of melanophores. It is notable that prolonged exposure to 1 background or lowering the temperature slows background adaptation. The former effect is probably due to changes in overall pigmentation of the minnow which masks the physiological changes whilst changes in environmental temperature of poikilothermic animals are likely to affect metabolism in the effector cells[7]. Too brief an exposure to 1 background (Figures 2 and 4), whilst allowing nervously coordinated responses to occur, fail to allow the circulating pituitary hormone titres to adapt to the new environment[5]. Accord-

1 G. H. PARKER, *Animal Colour Changes* (Cambridge University Press, London and New York 1948).
2 H. WARING, *Colour Change Mechanisms of Cold-Blooded Vertebrates* (Academic Press, New York and London 1963).
3 D. SLOME and L. HOGBEN, S. Afr. J. Sci. *25*, 329 (1929).
4 A. V. HILL, J. L. PARKINSON and D. Y. SOLANDT, J. exp. Biol. *12*, 397 (1935).
5 E. G. HEALEY, J. exp. Biol. *28*, 297 (1951).
6 E. G. HEALEY and D. M. Ross, Comp. Biochem. Physiol. *19*, 545 (1966).
7 U. WYKES, J. exp. Biol. *15*, 363 (1938).

ingly, transfers from black to white after a brief stay on black may be accelerated by the continued presence of pituitary paling hormone in the blood. In Figure 5, the relation between MI of the minnow, taken from HEALEY[5], and DOI, taken from comparable fish in the present study, is plotted, and considerable variation is found. This is partly because different groups of melanophores in the minnow skin react differentially to endogenous paling stimuli[5,8]. However, the variation observed in groups of fish changing shade under the same conditions represented in Figures 1–4 is small. The simple recording technique can be adopted for comparison of melanophore responses provided the temperature is known and is above 10 °C, and provided the previous chromatic history of the fish are known. Figure 6 shows dose response curves of black-adapted minnows injected with the catecholamines adrenaline or noradrenaline which bring about paling of the skin. The drugs are almost equipotent, when doses are expressed in terms of moles. Reports on further pharmacological studies on minnow melanophores, using the DOI standard, are in preparation.

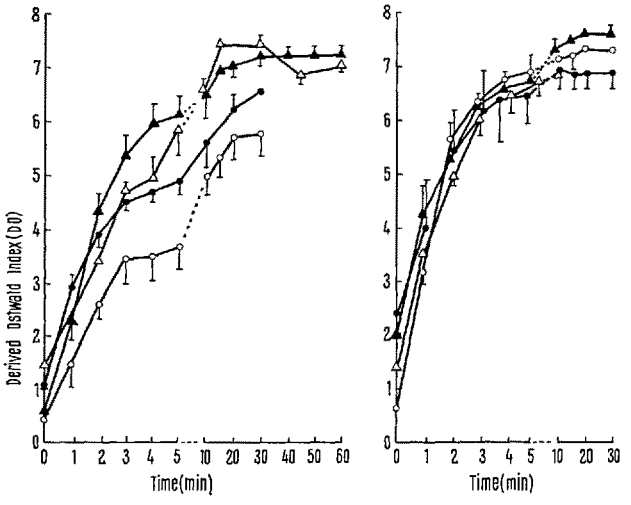

Fig. 1. Minnows darkening on a black background after a prolonged stay on a white background. o, After 2–3 years on white (n = 11; 20–22 °C), ▲, after 3 months on white (n = 9; 20–22 °C), △, after 3 months on white (n = 25; 15–16 °C), ●, after 3 months on white (n = 5; 10 °C). Vertical bars indicate the magnitude of the standard error at that point.

Fig. 2. Minnows darkening on a black background after a brief stay on a white background. o, After 1 h on white (n = 10; 20–22 °C), ▲, after $^1/_2$ h on white (n = 12; 20–22 °C), △, after 75 min on white (n = 7; 15–16 °C), ●, after $^1/_2$ h on white (n = 4; 10 °C).

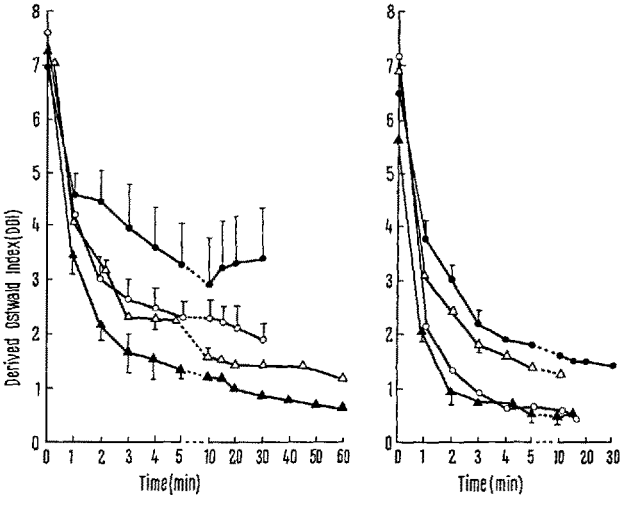

Fig. 3. Minnows paling on a white background after a prolonged stay on a black background. o, After 1 year on black (n = 12; 20–22 °C), ▲, after 3 months on black (n = 10; 20–22 °C), △, after 3 months on black (n = 10; 15–16 °C), ●, after 3 months on black (n = 5; 10 °C).

Fig. 4. Minnows paling on a white background after a brief stay on black. o, After 1 h on black (n = 10; 20–22 °C), ▲, after $^1/_2$ h on black (n = 11; 20–22 °C), △, after $^1/_2$ h on black (n = 18; 15–16 °C), ●, after $^1/_2$ h on black (n = 5; 10 °C).

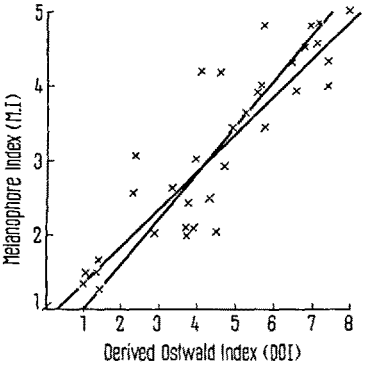

Fig. 5. Relationship between overall shade of minnows (taken from data in Figures 1 and 3) and the Melanophore Index of similar fish (taken from HEALEY[5]).

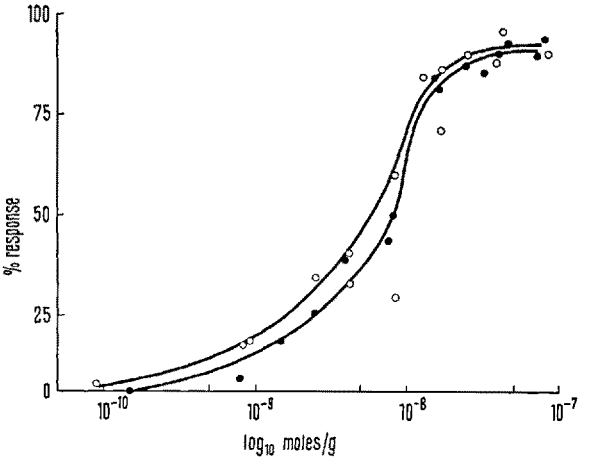

Fig. 6. Dose response curves obtained from adrenaline (●) and noradrenaline (o) injections in black adapted minnows, based on measurements obtained using the Derived Ostwald Scale.

Résumé. On décrit une technique simple pour mesurer les réactions mélanophoriques dans la peau des poissons.

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Marine Science Laboratories, Menai Bridge, Anglesey (U.K.), 29 April 1968.

[8] D. PYE, J. exp. Biol. *41*, 535 (1964).