Numbers of normal and abnormal fetuses (percentage in brackets) after 5 regimes

Group	Number of litters	Fetuses Normal	Resorbed	Exencephaly	Micropthalmia	Total	Litter size (1SD)
1 Normal diet (ND)	25	246 (94.6)	14 (5.4)	-	-	260	10.4 (± 1.96)
2 Deficient diet (DD)	24	214 (96.0)	9 (4.0)	-	-	223	9.3 (± 1.76)
3 ND + trypan blue (TB)	18	110 (59.8)	53 (28.8)	4 (2.2)	17 (9.2)	184	10.2 (± 2.07)
4 DD + TB	19	36 (20.9)	84 (48.8)	20 (11.6)	32 (18.6)	172	9.05 (± 2.09)
5 DD + TB + vitamin D	19	42 (23.1)	89 (48.9)	25 (13.7)	26 (14.3)	182	9.6 (± 1.84)

pellet diet but after timed mating were given a single i.p. injection of 1% aqueous trypan blue (British Drug Houses) on day 9 of pregnancy. The dose was 0.125 g/kg b.wt, a dose found by previous experimentation to produce few neural tube anomalies. The 4th group were fed the deficient diet and mated like group 2 but received a single i.p. injection of 0.125 g/kg of trypan blue on day 9 of gestation. The 5th group received the same treatment as group 4 but in addition were given 100 IU of vitamin D_3 orally each week to keep their serum levels of 25-hydroxycholecalciferol in the normal range.

After 3 months on the deficient diet serum levels of 25-hydroxycholecalciferol ranged from < 2.35–5.6 ng/ml compared with normal values of 15–18 ng/ml. On day 21 or 22 of gestation the rats were killed by chloroform, resorption sites recorded, and live fetuses dissected out and inspected for external malformation. For examination of the skeleton some embryos were cleared and stained with methylene blue or alizarin red. Histology of the limbs was carried out on other fetuses.

Results and discussion. The table shows that no external anomalies were found in either of groups 1 or 2. No skeletal defects were seen after staining with methylene blue or alizarin red, and the histology was also normal. The litter sizes were significantly smaller for animals on the deficient diet (Student's t-test, $p = 0.019$).

The dose of trypan blue that produced 2% exencephaly and 9% micropthalmia in the offspring of rats on a pellet diet (group 3) resulted in a statistically significant increased incidence of these defects (χ^2 with Yates' correction, $p = 0.0012$ for exencephaly, $p = 0.015$ for micropthalmia) when the mothers were on the deficient diet (group 4). The resorption rate was also increased. However, an oral supplement of vitamin D_3 had no protective effect on the incidence of malformation or resorption (group 4), although it did insignificantly increase the litter sizes.

The conclusion to be drawn is that the increased incidence of anomalies relates to some deficiency in the diet other than that of vitamin D. It also stresses the importance of an adequate diet during pregnancy particularly in the presence of known or unknown environmental teratogens. Fasting has previously been shown to be teratogenic in mice[9-11] and to enhance the teratogenic effects of cortisone[9]. In Britain the incidence of anencephaly and spina bifida has tended to be higher in the lower social classes[12] and thus it is interesting to speculate whether an inadequate diet, by enhancing the effect of some unknown environmental teratogen, might be one of the contributory factors in the embryogenesis of neural tube malformations.

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2 H. Kalter and J. Warkany, Physiol. Rev. *39*, 69 (1959).
3 G.A. Lumb, E.B. Mawer and S.W. Stanbury, Am. J. Med. *50*, 421 (1971).
4 E.B. Mawer, J. Backhouse, L.F. Hill, G.A. Lumb, P. de Silva, C.M. Taylor and S.W. Stanbury, Clin. Sci. molec. Med. *48*, 349 (1975).
5 J.O. Davies, Biol. Bull. *87*, 73 (1944).
6 C.O. Carter, J. biosoc. Sci. *1*, 71 (1969).
7 T. Suda, H.F. de Luca and Y. Tanaka, J. Nutr. *100*, 1049 (1970).
8 Publication 990 National Academy of Sciences, National Research Council, Washington 1962.
9 H. Kalter, Proc. Soc. exp. Biol. Med. *104*, 518 (1960).
10 M.N. Runner and J.R. Miller, Anat. Rec. *124*, 437 (1956).
11 R.D. Hemm, L. Arslanoglou and J.J. Pollock, Teratology *15*, 243 (1977).
12 K.M. Laurence, C.O. Carter and P.A. David, Br. J. prev. soc. Med. *22*, 212 (1968).

Presence of melanosome-like granules in dermal erythrophores of the tropical teleost *(Badis badis)*

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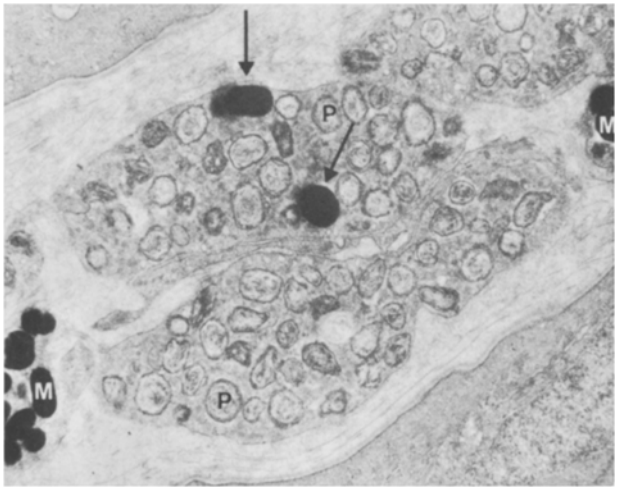
Summary. Melanosome-like granules were found in dermal erythrophores of the adult tropical teleost, *Badis badis*. Possible mechanisms of their formation are discussed as compared with previously reported examples of the hybrid chromatophore.

Chromatophores of lower vertebrates normally have 1 specific type of pigment organelles, which is characteristic for the cell type. Melanosomes are specific to melanophores, pterinosomes to xanthophores or erythrophores, and reflecting platelets to iridophores. In some cases, however, examples of the hybrid chromatophore[3] have been reported which have pigment organelles of different types in a single cell. At light microscopic level, erythrophores containing melanin granules or both melanin granules and guanine crystals have been reported in integuments of the frog[4]. At electron microscopic level, xanthophores or erythrophores containing melanosomes have been reported in scales of the iguanid lizard[5], integuments of the red-backed salamander[3], and fins of the zebra danio[6]. Iridophores containing melanosomes have been reported in the iris of the Inca dove and the Mexican ground dove[7]. Xanthophores and iridophores containing premelanosome-like inclusions have been reported in integuments of the young

chameleon[8]. Melanophores containing reflecting platelets have been reported in the tapetum lucidum of the stingaree[9] and integuments of the tree frog[10]. Erythrophores containing reflecting platelets have been reported in integuments of the ribbon snake[11]. Very little is known, however, about mechanisms of formation of the hybrid chromatophore.

The present study reports the presence of erythrophores containing melanosome-like granules in the dermis of the tropical teleost, *Badis badis*. The features of the hybrid chromatophore in this teleost and possible mechanisms of its formation are discussed.



Melanosome-like granules (arrows) in the process of a dermal erythrophore of the tropical teleost *(Badis badis)*. M: melanosome in neighboring melanophore; P: pterinosome. × 10,000.

Materials and methods. The dermis and dorsal fins were isolated from adults of *Badis badis* in Ringer's solution and cut into small pieces in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. They were prefixed with the same solution for 2.5 h at room temperature, and then postfixed with 1% osmium tetroxide in 0.1 M cacodylate buffer for 1.5 h at 4 °C. In some cases, they were fixed for 1 h with the cold mixture of 2.5% glutaraldehyde and 1% osmium tetroxide in 0.1 M cacodylate buffer containing 0.02% $CaCl_2$, pH 7.4. After dehydration through the ethanol series, specimens were infiltrated in Spurr medium[12] under vacuum and then polymerized. For light microscopy, 1 µm sections were cut and stained with the mixture of methylene blue and azure II[13]. For electron microscopy, ultrathin sections were cut with an LKB ultramicrotome, stained with uranyl acetate and lead citrate[14], and examined with a Hitachi Hu-11-DS electron microscope at 75 kV.

Results and discussion. Badis badis can change their body color rapidly, depending on their surroundings. The bar patterns on their body surface consists of alternate stripes of black and red. By detailed observations with binoculars, black stripes almost consisted of melanophores, but there was a fair sprinkling of melanophores besides erythrophores in red stripes. Near the boundaries of the 2 stripes, melanophores and erythrophores were intermingled and often contiguous to each other. By light microscopic observations, melanophores and erythrophores seemed to have no other pigment granules than their own characteristic ones.

By electron microscopic observations, most of chromatophores had only their own characteristic pigment organelles. However, erythrophores containing highly electron dense granules, probably melanosomes, were found, although the frequency of appearance was very low. These melanosome-like granules were distributed randomly in erythrophores alone or making clusters of a few granules. Such granules were also found in the cytoplasm of dendritic processes (figure). It has been reported that pigment organelles having the intermediate features between 2 definitive forms have been observed in integuments of the red-backed salamander and the iris of the Inca dove and Mexican ground dove[3,7]. However, no such intermediate organelles were found in the chromatophores of *Badis badis*. The hybrid chromatophore has often been found where different types of chromatophores are contiguous[3,7,9,11,15]. In *Badis badis*, chromatophores of different types were often contiguous each other, but only few of the erythrophores had melanosome-like granules.

Mechanisms of the formation of the hybrid chromatophore are unknown. The 1st explanation for it may be that the hybrid chromatophore is formed by production of different types of pigment granules (chromatophore metaplasia)[11] or by transformation of mature pigment granules of a certain type into another type (pigment organeller metaplasia)[3] in a single cell. The fact that iridophores isolated from bullfrog tadpoles transform into melanophores *in vitro*[16], seems to support the hypothesis of chromatophore metaplasia. Pigment organeller metaplasia have been postulated to explain the presence of the intermediate organelles of 2 types of pigment granules[3,7]. In the case of the red-backed salamander, however, the intermediate forms between pterinosomes and melanosomes could be regarded as showing a certain developmental stage of pterinosomes[17]. The 2nd explanation for the formation of the hybrid chromatophore may be that pigment granules of different types are acquired by phagocytosis from their surroundings.

In the case of *Badis badis*, neither intermediate organelles nor phagocytosis was observed, although many melanophores and erythrophores were distributed contiguously to each other. Therefore, the presence of the hybrid chromatophore in the adult *Badis badis* seems to be explainable by chromatophore metaplasia. However, further studies on chromatophore formation during ontogeny of the *Badis badis* will be necessary to confirm this hypothesis.

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3 J.T. Bagnara and J.D. Taylor, Z. Zellforsch. *106*, 412 (1970).
4 W.J. Schmidt, Ergebn. Anat. Entw. Gesch. *58*, 643 (1920).
5 N.J. Alexander and W.H. Fahrenbach, Am. J. Anat. *126*, 41 (1969).
6 F. Kirschbaum, Wilhelm Roux Arch. *177*, 129 (1975).
7 W.R. Ferris and J.T. Bagnara, in Pigmentation: its genesis and biologic control, p. 181. Ed. V. Riley. Appleton-Century-Crofts, New York 1972.
8 N.J. Alexander, J. invest. Derm. *54*, 82 (1970).
9 H.J. Arnott, A.C.G. Best and J.A.C. Nicol, J. Cell Biol. *46*, 426 (1970).
10 J.D. Taylor, J. Ultrastruct. Res. *35*, 532 (1971).
11 J.T. Bagnara, in: Pigmentation: its genesis and biologic control, p. 171. Ed. V. Riley. Appleton-Century-Crofts, New York 1972.
12 A.R. Spurr, J. Ultrastruct. Res. *26*, 31 (1969).
13 K.C. Richardson, L. Jarett and E.H. Finke, Stain Technol. *35*, 313 (1960).
14 E.S. Reynolds, J. Cell Biol. *17*, 208 (1963).
15 J.D. Taylor and J.T. Bagnara, Am. Zool. *12*, 43 (1972).
16 H. Ide and T. Hama, Devel. Biol. *53*, 297 (1976).
17 M. Yasutomi and T. Hama, Z. Zellforsch. *137*, 331 (1973).