and 2.5% glutaraldehyde in phosphate buffer (pH 7.4). Dorsal root ganglia were then extirpated from the cervical region. These samples were postfixed in phosphate-buffered 2% osmium tetroxide, and then dehydrated in a graded series of ethanol and embedded in Epon 812. Ultrathin sections were stained with lead citrate and examined in a Zeiss EM 9S electron microscope. The sectional area of individual mitochondria which were fixed with 6.25% glutaraldehyde was planimetrically measured and statistically analyzed. Mitochondria from other nerve cells on the same section were used for control measurements.

The unusual ganglion cells were found in 4 rats out of 10. 3 of these 4 animals were prefixed with 6.25% glutaraldehyde, and 1 of them with a mixture of 2.5% formaldehyde and 2.5% glutaraldehyde. Unusual ganglion cell is smaller and its cytoplasm is darker. The nucleus, Golgi-apparatus and ergastoplasm seem to be normal. There is a moderate increase in the number of lipofuscin-granula. The unusual ultrastructural characteristics in these cells are observed in the mitochondria which are randomly distributed through the cytoplasm (Figure 1). Two varieties of mitochondrial types which will be designated as types 1 and 2 are clearly discernible.

Type 1 mitochondria. These mitochondria are generally ovoid. The mean value of sectional area is 2.0 μm^2 with a standard deviation of 0.8 μm^2 ($n = 174$). This type of mitochondria is as volume about 8 times greater than the control mitochondria (0.5 μm^2, S.D. = 0.3 μm^2, $n = 164$). These mitochondria have tubulo-saccular cristae[8] which vary in diameter from 50 to 100 nm. The tubulo-saccular cristae are frequently accompanied by circular-arranged cristae (Figures 2 and 3). The tubulo-saccular cristae may be observed at the edge of the mitochondrion. In the matrix of the central region, various densities of intramitochondrial inclusions, up to 250 nm in diameter, are often observed. The intracristal spaces are either slightly electron dense or lucent. Frequently rod-shaped electron dense substances, about 6 nm in diameter, are seen in the dilated cristae (Figure 2). A most striking phenomenon is that the matrices show more electron dense than those of the control mitochondria. The mitochondrial outer membrane may be occasionally interrupted with the stalks of saccular cristae which seem to be in direct contact with the cytoplasm (Figure 2).

Type 2 mitochondria. These mitochondria are round. The mean value of sectional area is 2.8 μm^2 with a standard deviation of 1.1 μm^2 ($n = 78$). This type of mitochondria is as volume about 70% greater than type 1 mitochondria. When cristae are present, they seem to be normal (Figure 3). But some of these mitochondria are depleted of cristae which are replaced by an amorphous or finely filamented matrix. The matrix is not electron dense as that of the type 1 mitochondria. As in the type 1 mitochondria, various densities of intramitochondrial inclusions and intracristal substances are observed.

On a rare occasion, a mixed type of mitochondria was found as well (Figure 1). These contained both normal and tubular cristae. The matrices were dense as those of the type 2 mitochondria.

The question has to be raised whether the unusual phenomena described are a fixation artefact. However this seems unlikely since no mitochondrial changes were noticed in other nerve cells which were fixed through perfusion at the same time. The specific origin and function of these mitochondria is not known, but one might hypothesize that degenerative or ontogenetic changes of mitochondrial enzymes could have altered their inner structures[8,9]. The unusual intramitochondrial inclusions here described might be considered as accumulations of mitochondrial metabolites. The mixed type of mitochondria could not be found frequently, however it may be possible that one of these types of mitochondria can change to the other type. It is also noteworthy that the ganglion cells with atypical mitochondria do not contain any normal mitochondria. This might indicate that the ganglion cells with the atypical mitochondria perform a different function.

Zusammenfassung. In den Spinalganglien der Ratte wurden Zellen mit auffallend stark veränderten, sehr voluminösen Mitochondrien gefunden, deren Funktion jedoch unbekannt ist.

Y. HAYASHIDA[10]

II. Anatomisches Institut der Ruhr-Universität Bochum, D-4630 Bochum (Germany), 19 September 1972.

[1] S. L. PALAY and G. E. PALADE, J. biophys. biochem. Cytol. *1*, 69 (1955).
[2] K. H. ANDRES, Z. Zellforsch. *60*, 633 (1963).
[3] E. B. MASUROVSKY, M. B. BUNGE and R. P. BUNGE, J. Cell Biol. *32*, 497 (1967).
[4] C. SOTELO and S. L. PALAY, Lab. Invest. *25*, 653 (1971).
[5] K. H. ANDRES, Z. Zellforsch. *55*, 1 (1961).
[6] J. CERVÓS-NAVARRO, Arch. Psychiat. NervKrankh. *199*, 643 (1959).
[7] M. B. BUNGE, R. P. BUNGE, E. R. PETERSON and M. R. MURRAY, J. Cell Biol. *32*, 439 (1967).
[8] E. LINDNER, Z. Zellforsch. *72*, 212 (1966).
[9] R. LUFT, D. IKKOS, G. PALMIERI, L. ERNSTER and B. AFZELIUS, J. clin. Invest. *41*, 1776 (1962).
[10] Present address: Department of Physiology, Osaka City University, Medical School, Asahimachi, Abenoku, Osaka 545 (Japan).

Hormonal Induction of Ovulation and Spawning in the Blind Cave Fish, *Anoptichthys jordani* with the Use of Human Chorionic Gonadatropin

The Mexican blind cave fish, *Anoptichthys jordani*, discovered and named by HUBBS and INNES in 1936, members of the family Characidae, are characterized by atrophy of the eyes, little pigment and solitary negative phototactic behaviour[1,2]. These fish offer investigators a potential model with which to study some of the questions of evolution, development and specifically, gene expression concerning the structure and function of the eye and its lens.

Since their discovery, this species has been the subject of investigation by behaviourists, morphologists and geneticists[3–5]. Because breeding these fish naturally in aquaria has proven difficult in the past, these studies were limited mostly to adult fish. Increasing interest, however, in this species is reflected in the literature[2,6,7]. Although CAHN[1] and GERTYCHOW[2] found that obtaining natural spawns of *A. jordani* under laboratory conditions was

[1] P. H. CAHN, Bull. Am. Mus. nat. Hist. *115*, 69 (1958).
[2] R. GERTYCHOWA, Folia biol., Kraków *18*, 9 (1970).
[3] C. M. BREEDER, Trans. New York Acad. Sci. *5*, 158 (1943).
[4] C. M. BREEDER, Zoologica, N.Y. *26*, 123 (1941).
[5] C. M. BREEDER, Copeia *1*, 26 (1943).
[6] P. SADOGLU and A. MCKEE, J. Hered. *60*, 10 (1969).
[7] S. WHITT and S. MAEDO, Biochem. Genet. *4*, 727 (1970).

difficult and infrequent, especially during the winter months, and the percentage of fertilized ova and surviving fry was low (an observation also noted by this author), they point out that *A. jordani* is the only cave fish capable of prolonged survival and reproduction under laboratory conditions.

The artificial induction of spawning by the injection and implantation of pituitary glands from freshly killed carp was attempted during the winter months by CAHN[1]. She reported that many females became abnormally enlarged by this treatment but failed to spawn. Therefore, a reliable method of inducing ovulation and spawning in these fish in order to provide sufficient embryos of *A. jordani* for investigation was pursued by this author. Chorionic gonadotropin, for several years successful for this purpose in certain amphibia, e.g. *Xenopus* and *Bufo* (for references see[8]) was used.

Materials and methods. 26 adult blind Cave Fish, 3.5–5 cm, were purchased from a local pet shop. They were placed (in groups of 5) in 20 to 35 l stock aquaria. The pH of the water was maintained at 7.0 and the temperature between 24 °C and 27 °C. Their diet consisted of commercial flake food, frozen brine shrimp, grated beef and *Tubifex* worms.

Since these fish are egg-scatterers and eat their own eggs, spawning tanks were constructed that contained a nylon net (3 mm hole size) suspended by a plastic frame and held approximately 3 inches above the bottom of the bare tank by a series of plastic trays, intended to catch the adhesive eggs. Nothing else was in the tank except a charcoal type filter (Figure).

Six experiments were conducted during the winter months. In the first, 1 male and 1 female were injected i.m. with 50 international units (IU) of human chorionic gonadatropin (HCG-Rugby Laboratories, Inc. Inwood, Long Island, N.Y.) in 0.05 ml. of the supplied diluent and placed in a spawning tank. The 2nd consisted of 1 male, injected with 50 IU of HCG and an uninjected female. The 3rd, comprised 1 female injected with 50 IU of HCG and 1 uninjected male. In the 4th experiment, 4 male and 4 female fish were removed from the stock aquaria and isolated in 8 one-gallon plastic trays for 1 month. They were then placed together in a 75 l spawning tank to determine if these conditions would produce natural spawning. These fish were allowed together for 1 week. No spawning occurred during this time, however. 2 pair were then randomly chosen from the 8 fish in experiment 4, injected with 50 IU of HCG and returned to the spawning tank for the 5th experiment. The remaining 2 pairs were removed to another spawning tank to serve as controls. Matched controls were simultaneously arranged in the other 4 experiments and where appropriate, given 0.05 ml of diluent. The 6th experiment employed 4 injected males and 2 injected females, in a 75 l spawning tank, for the purpose of obtaining embryos for other investigations. No control was established. All injections were made with a 30 gauge needle under MS-222 (Tricaine Methanesulphonate-Sandoz) anesthesia at a dilution of 1:10,000 in aquarium water.

Results and conclusions. In the 1st experiment, at 6 h post-injection, 150 eggs were shed, and about 15% were viable. In the 2nd experiment, after about 4 h the male exhibited mating behaviour which persisted for 4 h but no eggs were shed by the female. In the 3rd experiment, intermittent courtship was noted after 6 h with no spawning. The female was found shedding eggs 20 h later, none of which developed. The 4th experiment did not result in a spawning; but those removed and injected for the 5th experiment, spawned 6 h post-injection. Approximately 2000 eggs were shed and about 800 were viable. In the 6th experiment, spawning began within 6 h, yielding approximately 1000 eggs, 65% of which were viable. In experiments 1 through 5, the controls did not spawn nor exhibit any spawning behaviour.

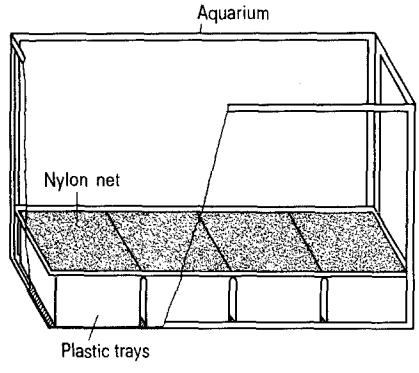
The results of these experiments indicate that both the male and the female must be injected with HCG for the induction of ovulation to result in a fertilized spawn. In addition, injection of more than 1 pair under the conditions of experiment 6 (which has since been repeated with similar results) increases the yield and percent of viable embryos. These Characins are at present commercially bred in pools in Florida, where the sun light, temperature and water conditions favor their reproduction. The viability nontheless of spawns under these conditions is reported to be low (approximately 50%)[9].

The use of HCG offers investigators, especially those interested in development, a fairly reliable method for inducing sexual behaviour, ovulation and spawning in intact *A. jordani*. Preliminary results (unpublished) have also shown that HCG is effective in other members of Characidae (i.e., *Hemigrannus caudovittatus*) and its general use in obtaining embryos of other fish should be investigated[10,11].

Zusammenfassung. Experimentell wird gezeigt, dass die Injektion von menschlichem Choriongonadotropin beim Höhlenfisch *Anoptichthys jordani*, das Fortpflanzungsverhalten vermehrt und der Ertrag an entwicklungsfähigen Eiern grösser ist, wenn mehrere Pärchen gleichzeitig mit Hormon behandelt und im gleichen Aquarium zur Eiablage belassen werden.

S. M. ZEITLIN

School of Veterinary Medicine,
University of Pennsylvania, 3800 Spruce Street,
Philadelphia (Pennsylvania 19104, USA),
1 August 1972.



Typical spawning tank (75 l) devised and used in these experiments (description in text).

[8] R. RUGH, *Experimental Embryology* (Burgess Publishing Co., Minneapolis, Minnesota USA 1962), p. 91.

[9] R. SOCOLOF, Ross Socolof Fish Farms, Inc., Bradenton, Florida, personal communication (1972).

[10] The author is grateful to Dr. D. S. MCDEVITT for many worthwhile discussions, advise and laboratory space.

[11] This investigation was supported in part by the Department of Anatomy, School of Veterinary Medicine, University of Pennsylvania; and by Grant No. NSF-1G-70-11 to the University of Pennsylvania (Dr. D. S. MCDEVITT).