The Axolotl Colony at Indiana University

Rufus R. Humphrey, Department of Zoology, Indiana University

I. Early History; the colony at Buffalo, New York.

The early history of the axolotl colony at Indiana University is that of the axolotl stocks of R.R. Humphrey in the Anatomy Department of the University of Buffalo (now a part of the University of the State of New York).

In Humphrey's earlier experimental studies on amphibian embryos begun in 1924 at Buffalo, embryos of wild species collected locally were utilized (Ambystoma maculatum and Ambystoma jeffersonianum) or those of A. maculatum and A. tigrinum obtained from Tennessee or from the Chicago area. The first axolotl embryos used were obtained from the Effingham B. Morris Biological Farm of the Wistar Institute near Morrisville, Pennsylvania, in 1934. These were used for transplantation of the gonad-forming area into A. tigrinum or A. maculatum recipients, or as recipients for such transplants. Some of the surviving axolotls, all of the dark or wild type, were reared and some matings undertaken in 1935-1936.

The first mutant white axolotls in the colony were obtained from the Morris Biological Farm in the summer of 1935. The decendents of these animals are now commonly referred to as being of the Wistar strain, to distinguish them from white axolotls of other origins. The axolotl stocks at the Biological Farm were under the supervision of Dr. R.C. Hutchinson, who later became a member of the Anatomy Department of Jefferson Medical College in Philadelphia. He had been given some white axolotls by Dr. Ross Harrison who had brought them to Yale University from the University of Cracow in Poland. Dr. Hutchinson had obtained his first spawning from these white animals in the spring of 1935; the young from this spawning were several months of age by the time of Humphrey's visit to the farm in midsummer, and were large, vigorous larvae. The five given Humphrey were put in thermos jugs for the return trip to Buffalo. This trip not being by the most direct route, the axolotl passengers may be the only ones of their species to have had the distinction of visiting Fort Ticonderoga and ascending to the summit of Mount Washington. A pair of these white animals produced offspring in the following year, and from these are decended the white axolotls in many institutions around the United States. It is possible this white stock is represented in other countries as well, since white animals from the colony at Buffalo or Indiana have been taken or sent to Canada, Norway, France, Belgium, Holland, and Mexico. Some of them helped to restore the stock at the Wistar Institute Colony after it had been depleted badly by a virus infection in the late 1930's.

The original dark or wild type stock at the Morris Biological Farm was supplemented in the late 1930's by importation of animals from Mexico. Some young animals of this new strain were sent to Buffalo. It is recorded that they were of a darker color than the wild-type axolotls already in the colony, but it is uncertain whether they were melanoid animals of the genetic make up (m/m) later found in animals imported by DeLanney. These new dark animals, when mated with other dark animals in the Buffalo colony or with white animals, produced offspring which invariably metamorphosed before reaching sexual maturity. The pure darks of this new stock, if mated together, always produced neotenous young. These wild-type animals were found inferior as breeding stock to those of other dark strains, however,

and were completely eliminated from the colony at Buffalo before its transfer to Indiana in 1957.

A somewhat later addition to the wild-type stock at Buffalo came from Dr. Myron Gordon, then at the Museum of Natural History in New York. He had obtained some animals from Mexico, and sent a few to Buffalo in gratitude for white animals earlier sent to him. This dark stock proved very prolific and was maintained for several years. Some animals brought to Indiana University in 1957 were of this stock, but it has since been entirely replaced by other dark strains.

When, after the beginning of World War II, Dr. Johannes Holtfreter, interned in Canada as an "enemy alien", was released to resume his scientific career at the University of Rochester, axolotls from the stocks at Buffalo were given to him to help establish his colony there. Later, Dr. Holtfreter obtained from Dr. E. Caspari, then at Cold Spring Harbor, some descendents of animals which Caspari had imported from Europe. Most of these had been procured for him by Dr. Hans Gloor, of Zurich University from various European dealers, and were, as Gloor states, "of an entirely obscure origin." A few young animals of this imported dark stock were given to Humphrey by Dr. Holtfreter in 1951. Descendents of these animals have been designated as being of the "Holtfreter strain." Several were brought to Indiana University in 1957 with other animals of the Buffalo colony, and for a time were the only dark or wild-type strain maintained. Gene o, the subject of several papers, was discovered in this strain after the colony was brought to Bloomington.

The establishment of a stock of axolotls at the University of Buffalo proved highly advantageous to Humphrey. His earlier studies on sex reversal in Ambystoma larvae of various species had demonstrated that an ovary could undergo reversal to a testis whether in females united in parabiosis with a male, or in animals in which an ovary and a testis had developed simultaneously following transplantation of a gonadal preprimordium at a tailbud stage. There remained the problem of mating animals with such reversed gonads when they were of wild species which metamorphosed and which ordinarily would mate and spawn only after hibernation. Since a axolotls would mate under laboratory conditions, they were ideal material with which to bring this study to completion. The two available color patterns in the axolotl also made it possible to be certain that the progeny from a grafted white animal came from its own germ cells and not those of a gonadal graft from the wild-type (dark) donor which had supplied the graft. Ten years after the first white axolotls were obtained, data were published showing convincingly that in the axolotl the female and not the male is the heterogametic sex (Humphrey, 1945). Later work by others has demonstrated the same pattern of sex determination for Pleurodeles and Xenopus.

Overlapping somewhat the period of Humphrey's studies on sex reversal was the long period of his collaboration with Dr. Gerhard Fankhauser of Princeton in studies on polyploidy (1941 to 1957). During this latter period especially the majority of the matings were crosses of white with dark animals, since these would help to distinguish the origin of either spontaneous or experimentally induced heteroploids. Such outcross matings, however, would fail to reveal mutant genes carried by the various dark strains introduced into the colony. This may explain the fact that no mutant genes were discovered in any of the dark strains included in the colony at different times before its removal to Bloomington. The white axolotls of the colony all being descended from a single white female, matings of sibs or other close relatives were unavoidable and it was not long before the linked genes f-g carried by this female made themselves

evident. Gene g being a lethal, this mutant gene pair was a cause for much concern until it was found that it was not sex-linked and could therefore not affect the sex ratios in spawnings obtained from sex-reversed animals. The linkage of f and g having a very low cross-over rate, the pair was first interpreted and reported (1948) as a single gene (f). The discovery that a second gene was involved was soon made but this finding, and a description of the maternal effect of gene f, were not published until after the colony had been brought to Indiana.

One other mutant gene (v, determining a vasodilation and other effects) was discovered in the Wistar white strain only a few months before the stock was moved from Buffalo. The first animal known to carry the gene was a sex-reversed male (a genetic W/W female) and the gene may have arisen by a mutation in one of his parents, since it has never been found in any animals other than his direct descendents.

When Humphrey reached retirement age in 1957, his office and laboratory space in the Anatomy Department of the University of Buffalo were needed for his successor and other faculty members. Dr. Robert Briggs of Indiana University, appreciating the potential value of the axolotl stocks for research in experimental embryology, suggested to Dr. T.W. Torrey, then Chairman of the Zoology Department, that Humphrey be invited to Indiana University. This suggestion meeting the hearty approval of Dr. Torrey, Humphrey was given faculty status as a Research Scholar, and space and facilities adequate for continuation of his own research. The axolotls, transported by truck,arrived at their new home in early August, 1957. Grants for the maintainance have been provided by the National Science Foundation and the U.S. Department of Public Health.

II. Expansion of the Axolotl Colony at Bloomington

The several additions to the axolotl colony at Indiana Univerisityare all worthy of mention, since each has brought into the colony one or more new mutant genes.

Shortly after the colony was established in Bloomington, Dr. Louis DeLanney, then at Wabash College was given some white larvae which became the foundation stock of the large colony he now maintains at Ithaca College. Dr. DeLanney was interested in obtaining other unrelated axolotls, and in 1961 procured several wild adults from Mexico City. He gave to Humphrey eggs from the first spawning obtained from this wild stock. These animals, reared and mated, contributed to the colony four mutant genes: m, for melanoid; x, a lethal; c, the cardiac lethal gene which has been so widely studied by Justus, Lemanski, and others; and sp (spastic), a gene affecting the central nervous system and so modifying motor control. Gene p (precocious death) was discovered in this stock by Tompkins at Princeton, but has never been found in the Indiana stock.

Dr. DeLanney has made two other valuable contributions to the axolotl stocks at Indiana University. On a visit to Holland in 1962 he obtained from Dr. P.D. Nieuwkoop of the Hubrecht Laboratory some animals of the dark (wild-type) stock maintained there. From a sib mating of a pair of these animals in 1963, ten larvae were sent to Indiana University. Matings of these animals revealed that the "Dutch strain" carried two recessive lethal genes. One, designated ut (for "Utrecht lethal") is an autonomous cell lethal resulting in death of homozygotes within a few weeks after hatching. The second mutant, designated by cl (for cleavage defect), produces no effect recognizable in animals of either sex, but causes defective cleavage and early death of all embryos from females homozygous for it.

On a later visit to Europe, DeLanney obtained some white animals of the stock maintained by Newth in London. From a mating of two of these, a spawning was obtained in April, 1968, and 36 embryos sent to Bloomington. One fourth of these failed to eat and grow; sib matings of the survivors a year or more later confirmed that this white stock carried a gene for a lethal trait. This gene has been designated l (for London lethal). Adults of this stock tend to be of smaller size than the whites of the Wistar stock, and to have a greater number of melanophores, giving them a somewhat bluish-grey color or a mottled or spotted pattern. This coloration appears to be dominant over the more purely white body color of the Wistar strain. White animals with a D/D ancestor in addition to an English strain d/d may become so heavily pigmented as scarcely to appear to be d/d in genotype.

Another contributor to the axolotl stocks of the Indiana colony is Dr. Ronald Brandon of Southern Illinois University, whose first gift was a shipment of about 90 embryos from a sib mating of wild-type axolotls. These animals had been reared by Brandon; their parents, owned by Dr. R.W. Reese of St. Edward's University in Austin, Texas, had been purchased in December of 1968 in Marketplace Xochimilco in Mexico City. The larvae sent to Bloomington were found to include mutants which died within a few weeks after hatching somewhat like the x/x in the DeLanney strain or the ut/ut in the Dutch strain, but having some distinct differences. Many of the survivors were reared, and random matings of these and their progeny have resulted in identification of heterozygotes. The gene is designed by b (for Brandon lethal). Spawnings obtained in 1976 included x/x and mi/mi mutants as well as b/b. These three lethals are readily distinguished by gill type, and other features.

The second contribution by Dr. Brandon was a pair of adults which he had bought along with others at the Xochimilco market on May 21, 1970. Their age was undoubtedly over a year; the ragged tail fin of the male suggested many old injuries. Test matings showed that he carried none of the six mutant genes found in the Tompkins stock which also had come directly from Mexico City. No new mutants have been found in any of his descendents. The female was found to be heterozygous for gene q, earlier discovered in the Tompkins stock, and may have carried no other mutant genes. A test mating proved she did not carry genes h, st, and mi (see below).

Perhaps the largest contribution to the axolotl colony, at least from the standpoint of the number of mutant genes involved, is the six animals left in the colony by Dr. Robert Tompkins. He had received them from Dr. Rafael Palacios, a young Mexican scientist, sent to Bloomington by Dr. Saberon, Director of Mexico's National Institute for Medical and Biological Sciences, to observe the procedures used in rearing axolotls. Dr. Palacios admired the white animals Tompkins was rearing and was given a half-dozen of them; in return for these, six young dark animals were sent from Mexico City. Dr. Tompkins being unable to take these with him when he went to the University of Illinois for post-doctoral study, they were added to the Indiana University colony. Numerous matings ultimately revealed that each of the six carried one or more mutant genes, as follows: no. 1, male, genes as (ascites), ph (phocomelic), q (quivering), and st (stasis); No. 2, male, gene t (twisted gils, a lethal); No. 3, male, genes h ("hand" lethal), mi (microphthalmic, a lethal), and st (stasis); No. 4, male, only gene st; No. 5, female, only gene t; No. 6, female, only gene t. Animals 2,5, and 6, carrying this mutant gene only, were probably sibs. A mating of male No. 2 with female 5 produced offspring which have been inbred for a few generations without revealing mutant genes other that t.

A small but highly important contribution to the axolotl colony was made in 1963 by Dr. Joseph Gall who sent from Minneapolis an adult albino

tiger salamander which had been given him by a member of the Zoology Department of the University of Minnesota. Since hybrids of the tiger salamander with the Mexican axolotl were known to be viable and fertile, this albino female seemed to offer the opportunity to produce albino axolotls. The first step was to obtain offspring from the albino by artificial insemination of her eggs, using a sperm suspension from a white axolotl male. Only a few fertile eggs were obtained, and these were found to become abnormal and die as blastulae. The last one of these remaining alive was disaggreated and its cells used for nuclear transplants into white axolotl eggs whose own nuclei had been destroyed by ultraviolet radiation. In this way a clone of Mexicanum-tigrinum hybrids was produced. These soon showed abnormal development and a high mortality, and at early tailbud stages it appeared probable that few if any of them would survive. Their gonadal preprimordia (lateral mesoderm plus primordial germ cells) were therefore implanted orthotopically in white axolotl embryos of tailbud stages. Although the hybrid blastula furnishing the nuclear transplants was of genotype Z/W (female), one of the gonadal preprimordia, implanted in a male recipient underwent reversal and produced a functional testis. From this grafted animal, both albino and non-albino progeny were finally obtained, some white, some wild-type, some metamorphosing and some remaining neotenous. From albinos of neotenous type the various kinds of albino axolotls now available have been produced. These have been of inestimable value in research projects in which melanin pigment is an obstacle.

By repeatedly back-crossing albinos to pure axolotls and mating the progeny together to recover albinos, the tiger-salamander component in some albinos of the Indiana Colony has now been reduced to one-thirtysecond. Even when with far more of the tiger salamander in their makeup, however, the albinos now show no greater tendency to metamorphose than any of the various pure axolotl stocks.

III. Occurrence of Mutations in the Axolotl Colony

Although mutant genes have been identified in each of several dark (wild-type) strains, we have no proof that any of these have been the result of mutations in animals actually in the colony. The mutant genes have been discovered within a short time, often only one to three generations, after the stock was acquired. We have no information as to how many generations the ancestors of the Dutch and Holtfreter strains may have been in European laboratories. Their mutant genes (ut, cl, o) have never been reported by European scientists, and may be of fairly recent origin. The DeLanney, Brandon, and Tompkins stocks, on the other hand are so few generations removed from their Mexican habitat that it appears certain that their mutant genes were carried by the animals first brought from Mexico to the United States. We know this is true for all the mutants found in the Tompkins stock. Three mutant genes, q, x, and mi, have each been found in animals coming from Mexico at different times and to different persons. The one possible mutation of laboratory origin (nc, for non-cleaving) found in 1975, is at present represented by only one animal, now deceased. Pieces of ovary from this female were transplanted into several recipient albinos. Four of these now carry functional nc ovaries, demonstrated as such by numerous spawnings. We assume that nc is a recessive gene, and that it manifests itself only in the absence of cleavage in eggs of females homozygous of it.

The white strain of axolotl, in contrast with many of the dark stocks, has lived over a hundred years as a laboratory animal (it was first reported by Dumeril in 1870). The English white stock has undergone a modification in color, individuals often being so hyperpigmented as to appear gray or bluish-gray. This condition may be the result of a mutation. In any event, when the English white was crossed with whites of the Wistar strain, the progeny were heavily pigmented like the English parent. Aside from this possible mutation, the English

stock has only one known mutant gene, the lethal, l, recently described by Chung and Briggs.

The white stock obtained from the Wistar Institute in 1935 probably carried gene s (short toes) and the linked genes f-g at that time. It was not until 1957, 22 years later, that another mutant, v, was discovered in this stock. This was followed by the discovery of genes r (1958), y (1965), and e (1969). That each of these is the result of a new mutation is strongly supported by these facts: (1) each mutant was first found in a spawning resulting from a mating of closely related animals (sibs, mother and son, or father and daughter) and (2) every animal shown heterozygous for the gene had as a recent ancestor the one animal to which all heterozygotes could be traced, and (3) no animals other that the decendents of this one individual could ever be found to carry the gene. It seems highly improbable that a mutant gene determining such a conspicuous trait as the eyeless could have been present for over thirty years in a highly inbred stock without making its presence known.

The nucleolar mutants, so termed, have in all instances been found as viable white diploids among the otherwise dark offspring of D/D x d/d matings (non-viable white haploid or hypo-diploid progeny may also occur in such spawnings). The nucleolar mutant results from a change (mutation or deletion?) in one of the chromosomes from the dark parent carrying gene D and the nucleolar organizer. The resulting nucleolus being smaller than that in the homologous chromosome, all cells of the viable white are characterized by inequality of nucleolar size, sometimes so slight as to be readily overlooked, but sometimes, as in n^2, very marked. It seems possible (or even probable) that the first white animals appearing in Europe in 1870 may have arisen in like fashion from a dark embryo half of whose germ cells would have lacked gene D. Half the offspring of such an animal lacking D, a mating of two such progeny would result in a spawning including white animals (d/d).

Since the great majority of the mutants discovered in the axolotl are lethals, they are never represented in the colony by homozygous adults, but only by heterozygotes of normal phenotype. Test matings of young adults with known heterozygotes will continue necessary for each of these lethals if they are to continue available for further studies. The first mutant genes discovered in the colony, the linked f-g pair, were regarded as a nuisance and selection of breeding stock free of them was begun. The discovery of a viable f/f (a result of crossing over) stimulated interest in this gene pair, and led to the discovery of additional mutants. These now give the axolotl stocks of the colony their great value in embryological and genetic studies. Axolotls lacking mutants, of course, remain of great importance in many morphological and biochemical studies.

next issue: History of the Wistar Institute axolotl colony.