Table 2. Size (standard length, millimeters) of adult male platyfish with different genotypes.

Birth date	P^eP^e Y-*Ir* Y-*Ir*			P^eP^l Y-*Ir* Y-*Br*			P^lP^l Y-*Br* Y-*Br*		
	Mean	Range	*N*	Mean	Range	*N*	Mean	Range	*N*
				Pedigree 2828					
2/27/71	28		1	32	30–34	4			
				Pedigree 2918					
6/25/71	24.5	23–26	2	31	28–33.5	6			
9/30/71	22.1	19–25	5	27.3	24.5–30	2			
				Pedigree 3030					
2/15/72	25		1	29.3	28.5–31	3			
3/17/72	22.7	21–23.5	8	27.4	27–29	4			
				Pedigree 2964					
9/20/71				30.5	29.5–31	3	36.6	35–38	3
10/20/71				25.5	22.5–27	3	31.8	28–34.5	5
1/26/72 a				25.8	24–28	6	28.3	24.5–32	6
1/26/72 b				24.1	22–26	6	29	27.5–30.5	6
	X-Y-*Ir*			X-Y-*Br*					
				Pedigree 2974					
11/1/71	24.7	23–26.5	13	29.9	27.5–32	15			

P^l may specify two proteins differing in their efficiency in initiating morphogenic changes. The *P* locus probably exerts its effect directly on the pituitary gland, although the role of the hypothalamus should also be evaluated.

The *P* gene also has an indirect effect on the adult size of male platyfish. Since growth rate decreases with increasing androgen production (*9*), early maturing males are significantly smaller than late maturing ones. Size differences between the two classes of males were absolute within seven broods, whereas in one (9/30/71) the largest *IrIr* male surpassed the smallest *IrBr* male by 0.5 mm (Table 2). The exceptional *BrBr* male (2964-11) that matured at 18 weeks (Table 1), is also the smallest homozygous *Br* male obtained so far. Small and large fish grow at the same rate, but the former stop at an earlier age when they become sexually mature. Since these observations did not differ throughout the year, natural daylight, which was not controlled, cannot account for the differences in growth and age of sexual maturity.

The *P* factors have manifested themselves both in the offspring of intra- and interstrain crosses. This polymorphism is a natural component of wild populations and is apparently widespread. We have found it in platyfish stocks collected from four river systems (*10*). The sex chromosome constitution of the males, XY or YY, has nothing to do with adult size and age of sexual maturation. P^e and P^l can be both X- and Y-linked. An X chromosome with P^l has recently been identified from the Belize population (*10*).

Similar variations in size and age of sexual maturation have been reported for a number of other species of *Xiphophorus* (*11*), but little is known about the genetics of these differences. The polymorphism at this locus may have been important for the evolution of the genus, since various body parts show allometric growth and large males, for example, those of *X. pygmaeus*, not only assume a different habitus, but also develop structures not present in the smaller morphs (*12*). The allele for early gonadotrop differentiation has become fixed in *X. pygmaeus pygmaeus* inhabiting the Rio Axtla, and has led to a uniform population of small males. However, *X. pygmaeus nigrensis* in the Rio Choy is polymorphic at the *P* locus resulting in two kinds of males (*13*). Elmination of P^e from the Rio Choy would leave two populations: *X. pygmaeus pygmaeus* homozygous for P^e (small size) and *X. pygmaeus nigrensis* homozygous for P^l (large size), differing completely in breeding structure and appearance.

The *P* locus of *X. maculatus* is of general significance not only for pituitary gland function in teleosts, but for all vertebrates including man. This system may become an important model for studying the genetic control of endocrine structure and function.

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Perfluorocarbons Having a Short Dwell Time in the Liver

Abstract. *Perfluorinated organic liquids are useful as high capacity oxygen and carbon dioxide solvents. After intravenous infusion most of these perfluorinated emulsions are deposited in the liver and spleen in a matter of days, where they remain for the lifetime of the animal. Hence, while they may be useful as isolated organ perfusion media their value as artificial blood is limited. A family of perfluorocarbons has now been discovered, which, although deposited in the liver after circulation in the blood, leave the liver to be excreted via the lungs and skin in a matter of days without apparent harm to the animal.*

Since 1966, when organic liquid breathing was first reported, there have been over 100 publications concerning the use of inert fluorochemicals (*1*) in physiological research. These include reports of liquid breathing (*2*), of organ perfusion (*3*), of infusion in whole animals (*4*), and as radiographic

agents (5). A symposium held in 1969 gave the state of the art as of that time (6).

Most of these perfluorinated organic liquids are synthesized for industrial use because of their value as electrically nonconductive, chemically nonreactive, and heat stable liquid heat exchangers, leak detectors, and hydraulic fluids. Their high solubility for gases, a nuisance in commerce, is a highly desired property in biology. Including the isomers present there are hundreds of compounds of this general type, over a hundred of which we have studied.

Generally speaking, these perfluorinated compounds (PFC) are excellent solvents for a large variety of gases (7), are nonmetabolizable, and are almost completely insoluble in water. As a class they are very poor solvents for all but a few organic substances, and for these reasons can only be used for intravascular gas transport in the form of oil-in-water emulsions.

Efforts are being made in various laboratories to develop artificial blood, based on the use of oil-in-water PFC emulsions, where the emulsion is made with an emulsifying agent and mechanical energy. Such emulsions have remarkably low toxicity—we have 10 percent (by volume) fluorocarbon emulsions where the median lethal dose in mice after a single intravenous injection is 200 ml/kg, with the blood volume of the mouse being probably about 60 ml/kg. One of the main problems, if not the main problem, with the use of fluorocarbon emulsions has been that they are deposited in the liver and spleen, where they remain for the life of the animal. We now report the discovery of a number of closely related fluorocarbons that leave the liver and spleen of the mouse in a matter of days.

Emulsions are made with 5 percent Pluronic F68 in the water phase, with 10 percent by volume (about 19 percent by weight) PFC liquid and with the mixture being subjected to a brief sonication or to a pressure of about 550 kg/cm^2 in a Gaulin homogenizer. For any given surfactant concentration, each PFC has a characteristic rate at which it breaks down to particles when continuously sonicated and a characteristic point at which the particles can be made no smaller, as judged by optical measurements (Fig. 1). The emulsion was centrifuged to remove large particles, and each emulsion to be tested was injected intravenously into the tail veins of 200 albino Swiss mice at a rate of 2 ml/min. The mice were killed in groups at intervals thereafter, and their livers and spleens were analyzed for PFC by specific gravity, by direct combustion to fluoride ion with the use of sodium biphenyl (8), and often by hexane extraction and gas chromatography. The fluoride ion concentration was measured with an Orion lanthanum fluoride electrode. The PFC liquids were stoichiometrically converted to fluoride ion by the biphenyl method.

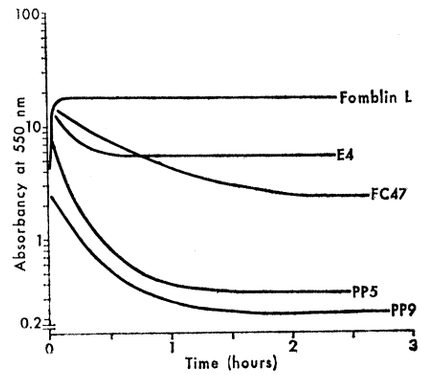

Fig. 1. Emulsion absorbance plotted against ultrasonication time. Emulsions were circulated through a Branson autotuned sonicator cell at 10°C, sample streams were flowed through spectrophotometer cuvettes having appropriate light paths, and the optical density was recorded. The optical density was calculated to account for the length of the cell path.

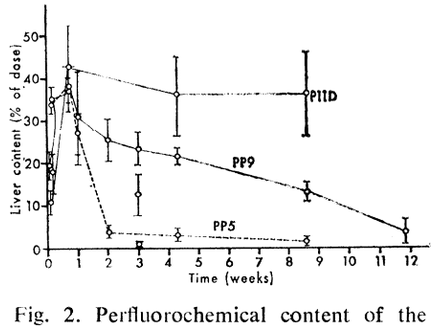

Fig. 2. Perfluorochemical content of the mouse liver plotted against time. The amount of PFC expressed as the percentage of initial dose remaining in the liver of Swiss albino mice injected with 10 percent PFC emulsion in 5 percent Pluronic F68 and killed at various intervals after injection is plotted. Groups of six mice were killed at 1 hour, 1 and 5 days, and 2, 3, 4, 8, 12, and 20 weeks. Livers and spleens were analyzed in duplicate for PFC by the sodium biphenyl procedure. Each point represents the average, and the vertical bars represent the standard deviation for six mice. Approximately 8 percent of the initial dose appeared in the spleen, and a similar rate of decrease in PFC was observed for PP5 and PP9.

Perfluorodecalin and perfluoromethyldecalin left the liver in a matter of days while P11D remained for months (Fig. 2). After 5 months the percentage of PFC of the injected dose remaining for P11D, PP5, and PP9 was 34, 2.3, and 1.3. We think that at least part of this residual PFC was due to impurities in the liquid. Other PFC tested, including FC75, FC47, P1D, E4, E3, Fomblin L, and Fomblin Z remained for months, probably for the lifetime of the animal. We still have dogs with apparently as much PFC in their livers as was there 4 years ago when the PFC was first administered.

Examination by gas chromatography and a ^{63}Ni electron capture detector of the breath of mice having PP5 or PP9 in their livers revealed the presence of enough of these substances to more or less account for their disappearance at the rate shown in Fig. 2. The breath of mice just injected with FC47, where the concentration in the blood was high (about 5 percent by volume), or from mice that had been injected months before, was analyzed by gas chromatography and no detectable PFC was found. The smallest amount of FC47 detectable with this instrument is about 200 pg.

In addition, we have found that perfluorodimethyldecalin perfluoro-(1,3-dimethyl)cyclohexane, perfluoromethylcyclohexane, and a mixture of perfluorodecalin and perfluoro-(1-3-dimethyl)cyclohexane leave the liver rapidly. In this unique family of perfluorinated decalins and cyclohexanes, those having the higher vapor pressures leave the most rapidly. It is easily demonstrated that these substances are present in the breath of mice and cats in large concentrations. Further, by placing a cup on the animals' skin, sampling the gas space, and analyzing by gas chromatography we can show that it is diffusing through the skin.

Analysis of the liver of a mouse which received (per kilogram of body weight) 100 ml of 10 percent FC47 (0.2 ml of FC47) 8 months earlier revealed the presence of 5.1 percent of FC47 (by volume). The liver was allowed to dry for 4 days over silica gel. Analysis of the gas above the silica gel showed no trace of FC47. The liver was removed, powdered in a mortar, and reanalyzed for FC47. It contained 8 percent PFC (by volume) per gram of dried liver. FC47 has a vapor pressure of only about 2 torr. But even P1D with a vapor pressure of 13 torr remained in the liver in-

definitely. The vapor pressure of perfluorodecalin is 14 torr and that of perfluoromethyldecalin is 5 torr.

Our results show that there are classes of PFC which form some kind of chemical bond with the liver substance. Such PFC contain atoms other than carbon and fluorine. All of them contain either a C-O-C or a C-N-C linkage. One possibility for such a coupling is that the unshared electron pair on the oxygen or nitrogen atom may passively form bonds with the substance of the liver. If the binding was to protein then it is surprising that it was not bound as much to, say, muscle. Ullrich's finding (*9*) that perfluorohexane is bound to, or at least interacts with, cytochrome P450 suggests that certain PFC may form complexes with certain iron-containing proteins. The straight-chain fluorocarbons studied so far, on the other hand, rapidly leave the liver of the intact animal.

Another possibility is that an active metabolic process is involved not only in the binding, but in the release from the liver. It may be, for example, that the perfluorocyclocarbons are actively excreted by the liver because they resemble steroid fragments.

This new family of compounds containing only carbon and fluorine and having cyclic structures may make possible PFC emulsions capable of being safely used in intact animals.

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1. The nomenclature for these compounds is not universally accepted. Perfluorochemical is used here to mean organic compounds that have been fluorinated until no hydrogen remains. Fluorocarbon refers to compounds having only fluorine and carbon in the molecule. PP5 is *cis*- and tris-perfluorodecalin and some impurities. PP9 is a mixture of isomers of perfluoromethyldecalin, some PP5, and some impurities. PP5 and PP9 are trade names of I.S.C. Chemicals, Ltd. P11D is a perfluorodiisopropoxybutane synthesized by the Allied Chemical Company. After this manuscript was read in proof we found that our perfluorodimethyldecalin (product 10964, PCR, Fla.) has the same infrared spectrum as the monomethyl compound.
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Impaired Learning and Decreased Cortical Norepinephrine after Bilateral Locus Coeruleus Lesions

Abstract. *Bilateral lesions of the nucleus locus coeruleus in rats deplete the cerebral cortex of norepinephrine and significantly diminish the rate of increase of running for food reward in a simple L-shaped runway. As assessed in this situation, learning was absent in those rats with the most complete ablations of the locus coeruleus, although these rats showed normal weight gain and normal motor and exploratory activity.*

Studies with the Falck-Hillarp histochemical technique (*1*) reveal the presence of a network of norepinephrine-containing nerve terminals in the mammalian cerebral cortex. Studies with lesions (*2*) and stimulation (*3*) show that these terminals are derived from cell bodies situated in the nucleus locus coeruleus in the floor of the fourth ventricle. Electrical self-stimulation can be obtained through electrode tips in close proximity to this nucleus (*4*), and this noradrenergic system may be one of two catecholamine-containing systems that will support this behavior (*5*, *6*). On the basis of theoretical considerations, it has been proposed (*5*, *6a*) that the norepinephrine-containing neurons arising from the cell bodies of the locus coeruleus function as a "reinforcement" system in the sense that this term is used in theories of learning.

Our experiments were designed to test the theory that the norepinephrine-containing neurons innervating the cerebral cortex form an essential component of the mechanisms of learning. Male hooded Lister rats (initial weight, 200 ± 10 g) were anesthetized with pentobarbitone, immobilized in a Kopf stereotaxic apparatus, and had burr holes drilled bilaterally in the skull over the cerebellum. In one group (BH) of six rats, no further procedures were carried out before the wound was resutured. In three further groups of rats, bilateral electrolytic lesions were made by passing a charge of 15 to 20 millicoulombs through the bare tip of a varnished steel electrode to an anal cathode. In one group (CB) of six rats, the electrode was located at symmetrically placed points in the cerebellar

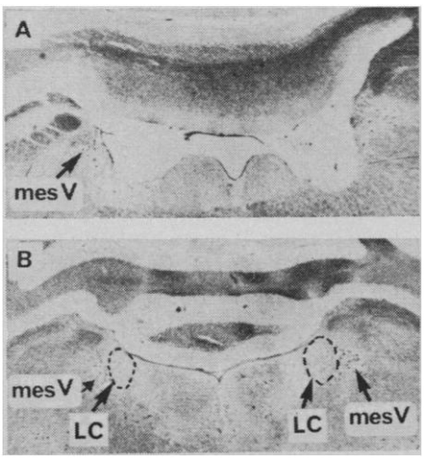

Fig. 1. Histological preparations of the region of the locus coeruleus in the floor of the fourth ventricle. (A) The locus coeruleus on each side has been ablated, although cells of the mesencephalic tract of the trigeminal nerve (*mesV*), situated laterally to the locus coeruleus, have been spared. This rat showed no increase in running speed in the course of behavioral testing. (B) The locus coeruleus (*LC*) is intact on both sides in a rat in group BH.