

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

<table>
<thead>
<tr>
<th>Birth date</th>
<th>( p^p )</th>
<th>( p^p )</th>
<th>( p^p )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Y-( l )</td>
<td>Y-( r )</td>
<td>Y-( l )</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>N</td>
</tr>
<tr>
<td>2/27/71</td>
<td>28</td>
<td>23-26.5</td>
<td>1</td>
</tr>
<tr>
<td>6/25/71</td>
<td>24.5</td>
<td>23-26</td>
<td>2</td>
</tr>
<tr>
<td>9/30/71</td>
<td>22.1</td>
<td>19-25</td>
<td>5</td>
</tr>
<tr>
<td>2/15/72</td>
<td>25</td>
<td>21-23.5</td>
<td>8</td>
</tr>
<tr>
<td>3/17/72</td>
<td>22.7</td>
<td>21-23.5</td>
<td>8</td>
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</tr>
<tr>
<td>1/26/72 a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/26/72 b</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

**Pedigree 2928**

**Pedigree 2918**

**Pedigree 3030**

**Pedigree 2964**

**Pedigree 2974**

**X-Y-\( r \)**

**X-Y-\( r \)**

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). Elimination of \( P \) from the Rio Choy would leave two populations: *X. pygmaeus pygmaeus* homozygous for \( P \) (small size) and *X. pygmaeus nigrensis* homozygous for \( P_1 \) (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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**References and Notes**


2. *Xiphophorus maculatus* is polymorphic for sex chromosomes. Three kinds of females (XX, WX, WY) and two kinds of males (XY, YY) may occur within the same natural population. YY males are not the result of special laboratory crosses as are the YY males of the rice fish, *Oryzias latipes* (T. Yamamoto, Genetics 58, 45 (1964)).


14. Supported in part by NIH grant 5 RO1 CA06665.

**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 fluorocarbons (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 iso-

mers 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 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/cm² 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 character-

istic point at which the particles can be made no smaller, as judged by optical measurements (Fig. 1). The emul-
sion 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.

Perfluorodecalin and perfluoromethyl-yldecalin 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 60Ni 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 perfluorodimethylydecalin perfluoro-(1,3-di- methyl)cyclohexane, perfluromethyl-cyclohexane, and a mixture of perfluorodecalin and perfluoro-(1,3-di- methyl)cyclohexane leave the liver rapidly. In this unique family of per-

fluorinated 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 perfluordecalin is 14 torr and that of perfluormethyldecalin 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. Ulrich'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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References and Notes

1. The nomenclature for these compounds is not universally accepted. Perfluorochrome 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. PPS is cis- and trans-perfluordecalin and some impurities. PFP is a mixture of isomers of perfluormethyldecalin, some PPS, and some impurities. PFS and PFP are trade names of E. I. du Pont. Perfluorodecalin, a fluorinated hydrocarbon synthesized by the Allied Chemical Company. After this manuscript was read in proof we found that our perfluoromethyldecalin (product 10964, PFR, Fl.) has the same infrared spectrum as the monomethyl compound.


8. Blood samples and tissue homogenates were pipetted directly into an excess of sodium biphényl reagent, where the fluorine on the compound was converted to fluoride ion; after dilution with buffer the F- activity was measured with an Orion electrode. The method is based in part upon that of P. P. Wohle and M. J. Faux [Anal. Chem. 38, 1971 (1966)].


10. Supported in part by grants HL12419 and HD05221 from the National Institutes of Health and a grant from the Southwestern Ohio Chapter of the American Heart Association. Dr. D. Steible and Mrs. D. Schwartz assisted with some of the analytical work. We have received gifts from all of the manufacturers of perfluorochemicals, and we are especially indebted to the Allied Chemical Company.

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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 millicolombs through the bare tip of a sharpened steel electrode to an anal cathode. In one group (CB) of six rats, the electrode was located at symmetrically placed points in the cerebellar cortex. In a second group of six rats, the electrode was located in the right side of the left hemisphere, at the level of the caudate nucleus. In a third group of six rats, the electrode was located in the right side of the right hemisphere, at the level of the caudate nucleus. In a fourth group of six rats, the electrode was located in the right side of the left hemisphere, at the level of the caudate nucleus. In a fifth group of six rats, the electrode was located in the right side of the right hemisphere, at the level of the caudate nucleus. In a sixth group of six rats, the electrode was located in the right side of the left hemisphere, at the level of the caudate nucleus.

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.

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