Temperature Dependence of Anesthesia in Goldfish

Abstract. *The anesthetizing concentrations in water of diethyl ether, chloroform, halothane ($CF_3CClBrH$), and methaxyflurane ($CHCl_2CF_2OCH_3$) have been determined for temperature-acclimated goldfish* (Carassius auratus) *at 5° to 30°C. At 20°C, the concentrations that anesthetize 50 percent of the fish are 2160, 167, 76.4, and 57.0 mg/liter, respectively; the corresponding partial pressures are 13.0, 3.36, 4.12, and 0.67 mm-Hg. The anesthetizing partial pressures increase with body temperature, with enthalpies of 8.6, 13.2, 12.5, and 12.3 kcal/mole, respectively.*

Pauling (*1*) has postulated that nonhydrogen-bonding anesthetics block transmission of nerve impulses by stabilizing the formation of hydrate microcrystals in the encephalonic fluid. Since the stability of hydrates decreases as the temperature rises, increase of brain temperature should raise the partial pressure of anesthetic required for hydrate formation and hence for anesthesia. Miller (*2*) has suggested that studies in poikilotherms be made to evaluate the relationship between temperature and anesthetizing partial pressure of volatile anesthetics. This report describes a quantitative technique for such studies and gives the initial results obtained.

As the test animal we selected goldfish (*3*) because they can be acclimated to a wide range of temperatures (0° to 41°C), they yield reproducible results, and they are inexpensive. Our fish were approximately 5 months old, 4 to 5 cm in body length, and 2.3 ± 1.2 g in weight. They were acclimated for at least 3 days in aerated tap water at the intended experimental temperature (± 0.5°C). The body temperature of small fish lies within 0.1°C of the water temperature (*4*).

For each trial, 10 fish were observed for 1 hour in a closed 4.24-liter anesthesia chamber, filled completely with anesthetic solution and immersed in water maintained to within ± 0.2°C. The chamber, a flat-sided aquarium jar with the top rim ground smooth to provide a flat sealing surface 8 mm wide, was sealed by a glass plate bearing an upright 30-mm inlet tube, 6 cm long, closed with a tight cork bearing a thermometer. The plate was held tightly to the rim by brass clips. Two pairs of 0.5-mm platinum wire electrodes (6 cm long, curved to arcs of a 9-cm circle, with leads sealed in glass tubes cemented through the plate), served to apply shock stimuli. One pair of electrodes extended 1 cm below the top plate and the other reached to 1 cm from the bottom of the chamber. A dry-cell discharge of 9 volts d-c, lasting 0.2 second, was passed across the 9-cm space between each pair of electrodes every 6 seconds, with a 3-second interval between shocks at the top and at the bottom. The current was approximately 15 ma; the polarity was reversed for alternate shocks.

We used reagent grade anhydrous ether (Mallinckrodt), spectrochemical reagent grade chloroform (Matheson), and clinical grade halothane (Fluothane-Ayerst) and methoxyflurane (Penthrane-Abbott). All anesthetics were protected from light and stored in the refrigerator. Care was taken to minimize handling losses of the highly volatile anesthetics, which were completely dissolved in concentrated solution before transfer to the anesthesia chamber. We filled a 250-ml flask to the top with tap water previously aerated for at least 16 hours, added a magnetic stirrer bar, and pressed out the excess water by momentarily inserting the 24/40 standard taper stopper. We then injected into the flask the calculated volume of chloroform, halothane, or methoxyflurane, measured at a temperature at which the density is known, using a microliter syringe (Hamilton No. 725-N or 750-N) with the needle tip kept well below the surface of the water. We replaced the stopper at once and let the mixture stir until solution was complete, which required about 2 hours. Ether concentrates were made by pipetting cold ether (0.7307 g/ml at 5°C) into ice-cold aerated tap water, leaving a 2-ml air space under the stopper to prevent loss of liquid ether when the stopper was inserted. The ether dissolved completely upon rapid stirring for about 15 minutes.

We placed the flask containing the concentrate in the thermostat until it came to the experimental temperature, replaced the glass stopper by a glass transfer tube, and emptied the 250 ml of concentrate swiftly (within 20 seconds) under the surface of 3.9 liters of fully aerated tap water in the anesthesia chamber, containing 10 fish. The chamber and inlet tube were immediately filled with water, leaving space only for the cork, and the tube was corked tightly. An adequate supply of dissolved oxygen in the sealed chamber was provided in the 183 ml of aerated water per gram of fish, which contained more than twice the maximum hourly oxygen requirement, even for fully active goldfish at 30°C, our most demanding temperature (*5*).

Addition of the anesthetic to the

Table 1. Relationship between temperature and anesthetizing partial pressure of volatile anesthetics in goldfish. The slope function S (*7*) is the average of the ratios AD_{84}/AD_{50} and AD_{50}/AD_{16}. (C, C_s, P_oX, P, and ΔH are defined in the text.) Anesthetic activities as defined by Ferguson (*12*) are equal to C/C_s. $\Delta H = [2.303R \log(P_2/P_1)]/(1/T_1 - 1/T_2)$, where R is the gas constant, and T the absolute temperature. The values of ΔH for chloroform apply at 10° to 20°C; the others apply at 10° to 30°C. Values of C_s and P_o for chloroform and ether are from the *International Critical Tables* or Landolt-Börnstein (*13*); for halothane they are calculated from data supplied by W. A. M. Duncan, Imperial Chemical Industries. For methoxyflurane, the P_o values are from data supplied by E. R. Larsen, Dow Chemical Co., and the C_s values were determined by one of us (J.F.C.).

Anesthetic and mole refraction [R]	Temp. (°C)	Slope function S	C (AD_{50}) (g/liter)	C_s (g/liter)	P_oX (mm Hg)	P (AD_{50}) (mm Hg)	ΔH (kcal/mole)
Diethyl ether	5	1.48	2.56	106	223	5.38	
[R] = 22.5	10	1.14	2.54	91.9	278	7.69	
	20	1.10	2.16	70.2	421	13.0	8.6
	30	1.19	1.85	54.1	615	21.0	
Chloroform	5	1.45	0.097	9.22	79.3	0.831	
[R] = 21.3	10	1.34	0.130	8.70	101	1.51	
	15	1.33	0.140	8.30	128	2.16	13.2
	20	1.26	0.167	7.95	160	3.36	
Halothane	5	1.45	0.0467	5.68	128	1.05	
[R] = 24.0	10	1.32	0.0640	5.23	157	1.92	
	20	1.35	0.0758	4.46	243	4.12	12.5
	30	1.27	0.0935	4.07	364	8.34	
Methoxyflurane	5	1.58	0.0243	1.83	9.2	0.122	
[R] = 27.2	10	1.18	0.0488	1.86	12.6	0.330	
	20	1.13	0.0575	1.94	22.7	0.673	12.3
	30	1.18	0.0719	2.00	39.0	1.40	

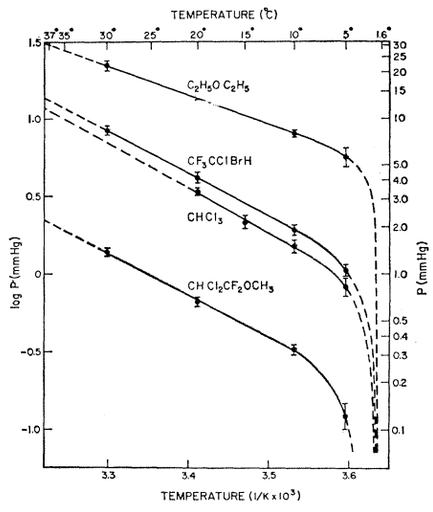

Fig. 1. Relationship between body temperature and partial pressure anesthetizing 50 percent of goldfish (*14*). Each point represents 50 to 190 fish. Vertical bars indicate the 95 percent confidence limits. The dashed lines below 5°C indicate that anesthesia occurs in 50 percent of the fish at 1.6°C in the absence of an anesthetic compound (P = O; log $P = -\infty$). The dashed lines above 30°C permit P to be extrapolated for comparison with values for other species. For example, the extrapolated values at 37°C resemble the anesthetizing partial pressures reported for mice, differing by a factor of 1.1 for ether (*12*); 1.8 for chloroform (*15*); and 1.04 for halothane (*15*).

chamber caused definite behavioral changes in the fish. Typically, they first displayed excitation, darting about the chamber and showing an increased rate and amplitude of respiration. After several minutes, activity and respiration began to decline and equilibrium was disturbed. Unanesthetized fish reacted to the shock stimulus by swimming away abruptly from the top and bottom shock regions and then avoiding those regions. Partially anesthetized fish reacted with a spasmodic twitch and uncoordinated swimming; they usually sank back into the bottom shock region, and often showed loss of the righting reflex. Anesthetized fish, by our criterion, were those fish that rested quietly in the shock regions and exhibited no activity beyond a slight twitch when shocked. Most anesthetized fish sank to the bottom, lying on their sides, but about 5 percent floated to the top shock region.

During the hour after adding the anesthetic, we made a triplicate count of the anesthetized fish every 3 minutes, allowing 30 seconds between the individual triplicate counts. There was recurrent waxing and waning of anesthesia in some fish. During the first 30 minutes the number of anesthetized fish gradually increased and reached a steady-state, after which no consistent rise or fall occurred (*6*). The 30 counts made from 32.5 to 60.5 minutes were averaged to determine the percentage of fish anesthetized. We calculated the AD_{50} (concentration that anesthetizes 50 percent of the fish) by the method of Litchfield and Wilcoxon (*7*). A small correction, which did not exceed 1.6 percent, was made for the estimated loss in transfer and by absorption of anesthetic into the fish (*8*).

We transferred the fish to fresh water after making the last count; recovery of normal swimming activity was usually complete within 5 minutes. The test fish were kept isolated for 24 hours. An average mortality rate of 0.7 percent in 1410 fish, with a maximum of 3.5 percent, was observed in the experiments with ether, halothane, and methoxyflurane at 5° to 30°C and with chloroform at 5° to 20°C. At 30°C, chloroform caused a mortality rate of 18 percent in 320 fish; the results with chloroform at this temperature and at 25°C were excluded as unreliable because of toxic effects.

The AD_{50} concentrations of chloroform, halothane and methoxyflurane did not exceed a mole fraction of 0.00003 or 3.6 percent of the saturation concentrations in water. At low concentrations, a linear relationship exists between the concentration of a dissolved volatile anesthetic and its partial pressure. We applied Henry's law to calculate the partial pressures at the AD_{50} concentrations in water, by the equation $P = (C/C_s)P_oX$, where P is the partial pressure (mm-Hg); C is the AD_{50} concentration (g/liter); C_s is the concentration of anesthetic in its saturated aqueous solution (g/liter); P_o is the vapor pressure of the pure liquid anesthetic (mm-Hg); and X is the mole fraction of anesthetic in its water-saturated solution; all at the experimental temperature. The mole fraction X of ether saturated with water at 5° to 30°C is 0.958 to 0.948. For the three halogenated anesthetics X exceeds 0.995, so that the partial pressure of the water-saturated compounds was considered equal to the vapor pressure of the pure compounds. The calculated partial pressures are listed in Table 1.

The curves in Fig. 1 show the dependence upon temperature of the partial pressure of anesthetic agent required to produce a fixed level of anesthesia. The ΔH values in Table 1 represent the overall enthalpy change per mole of anesthetic for whatever chemical reactions are involved in the anesthetic process (*9*). The observed fall in potency with rise in temperature is qualitatively in accord with the hydrate microcrystal theory and other theories of anesthesia but contrary to the Meyer-Overton theory. A strong argument for the latter was based on the parallel effects of temperature upon the oil-water distribution ratio and the potency of anesthetic agents (*10*). In the case of ether and halothane, however, this ratio rises with temperature (*11*) while potency falls. The technique described is also well-suited for synergism experiments (*9*) as suggested by Pauling (*1*).

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increased linearly from 155 at 10°C to 210 at 37°C. Published values for ratios at temperatures below 37°C are questionable for chloroform and not available for methoxyflurane. In general, a rise in temperature in the range 10° to 40°C causes a slight increase in the solubility of volatile compounds in oil and a decrease of their solubility in water, resulting in a rise in their oil-water distribution ratios.

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Endocrine Control of Tanning in the Crayfish Exoskeleton

Abstract. *Tanning of the newly formed exoskeleton of the dwarf crayfish,* Cambarellus shufeldti, *depends in part upon the presence of a substance from the eyestalk. Injection of eyestalk extract enhances tanning in a specimen with its eyestalks removed, whereas the exoskeleton of an untreated crayfish without eyestalks will not become as dark as that of an intact individual.*

The molting process in Crustacea has been reviewed by Passano (*1*). The eyestalks contain a molt-inhibiting hormone, and removal of the eyestalks has long been known to result in increased molting. The eyestalk is the site of important neurosecretory centers and contains the sinus gland, a neurohemal organ. After the old exoskeleton of higher crustaceans has been shed, hardening of the new one begins, inorganic salts and organic material being deposited. The organic matter participates in a tanning process that is the result of interactions between quinones and proteins (*2*). Fraenkel and Hsiao (*3*) recently showed that tanning in insects is regulated by an endocrine substance which is secreted by neurosecretory cells in the brain. This substance is neither the prothoracicotropic hormone nor the gonadotropic hormone. Insofar as we know, no one has investigated the possibility that tanning in crustaceans is controlled by a blood-borne substance. Previous investigations of hardening have centered on the control of deposition of inorganic materials after the molt.

The dwarf crayfish, *Cambarellus shufeldti*, undergoes the typical tanning process after molting. A golden brown pigment is produced in the transparent exoskeleton. The experiments described herein were undertaken to determine whether the tanning process in the dwarf crayfish is under endocrine control.

Specimens were collected near the town of Pearl River, Louisiana, in September 1963 when the population was undergoing its fall molt and in February 1964 during the spring molt. In the laboratory, specimens were kept individually in white enamelled pans under constant illumination, 1320 lumen/m^2, at 24° to 26°C. The color of the pleura, the downwardly directed lateral processes of the abdominal exoskeleton, was recorded daily with colored pencils. In this way any change in the amount of pigment deposited from day to day would be apparent. The pleura were chosen because they are thin and the recording of their color is not complicated by the presence of other colored tissues.

Both eyestalks were removed from each of a number of crayfish that were actively preparing for the molt. Seventy-five crayfish without eyestalks survived long enough to molt at least once. The average length of time required for molting to occur in these 75 crayfish was 6.5 days after the operation. After the molt, the newly formed exoskeleton of each crayfish was a very pale yellow, almost colorless. Gradually a small amount of golden brown pigment, less than in intact specimens, formed in each exoskeleton. Thirty of the 75 crayfish that had molted after eyestalk removal were kept as controls. Of these 30, 6 molted a second time. The average period between molts was 10.5 days. The remaining 24 control specimens died before they could molt a second time. The period of survival of the 24 averaged 6.6 days after the first molt without eyestalks. When the six specimens without eyestalks that molted twice during the experiment molted for the second time, the amount of pigment that had formed in their exoskeletons was less than the quantity that had been present just prior to the first molt.

To determine conclusively whether a substance in the eyestalk accelerates tanning, the 45 additional crayfish that molted after their eyestalks had been removed were injected with eyestalk extract on the day they molted and every second day thereafter. The eyestalk extract was boiled and centrifuged, and the supernatant was used for the injection. Boiling the extract eliminated the possibility that an enzymatic rather than endocrine process was involved. The dose per crayfish was one eyestalk extracted in 0.02 ml of physiological saline. Only 1 of 45 injected crayfish, as opposed to the 6 out of 30 controls, molted a second time. Presumably, this difference was due to the molt-inhibiting hormone present in the eyestalk extracts. The average length of survival of the injected crayfish that molted only once was 5.7 days after molting. As a result of the injections of eyestalk extract these crayfish produced the golden brown pigment in their exoskeletons at a faster rate than did the uninjected group, that is, they showed more tanning. Hence the conclusion that the eyestalk contains a substance that hastens tanning.

Rather than postulate a "tanning hormone" to account for these observations, we suggest that the molt-inhibiting hormone also controls tanning. However, the fact that a specimen without eyestalks will deposit some pigment in its exoskeleton after molting suggests an additional, extra-eyestalk source of the substance that promotes tanning. Stephens (*4*) has already found that the supraesophageal ganglia and circumesophageal connectives of a crayfish contain a substance that inhibits molting.

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