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Anesthesia and experimental methodology on fish

Anestesi og forsöksmetodikk på fisk

by

Torill Bergsjö

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(Norwegian Veterinary Journal)

The first part of the article summarizes the use of sedatives and anesthetics on fish. The requirements for these substances in the handling of fish are pointed out and a description is given of the effect of the sedative or anesthetic chosen and how it is administered. The following substances are described: MS-222 (Metacain), Quinaldine, methylpentynol, chloral hydrate, tertiary amyl-alcohol, 2-phenoxyethanol, chlorobutol, tribromomethanol, ether, carbon dioxide and urethane. p.313

The second part of the article describes the methodology which will most likely be of value in future studies in the field of fish pharmacology. The chapters are as follows: Basic principles of surgical operations on fish, blood sampling, measuring excretion products, tissue sampling, stomach content sampling by pumping and force-feeding with test substances.

The pioneering studies carried out by veterinary doctor Tore Håstein as a fish researcher has clearly shown the usefulness of veterinary-medical efforts in this field.

Fish rearing has perhaps as many veterinary-medical aspects as domestic animal rearing. At our institute we will therefore take up studies in the fields of fish pharmacology and toxicology.

This paper, which resulted from a competition announced last year, is a good beginning. The first experimental studies are under development at the institute.

We hope gradually to be of assistance to those who will spearhead developments in this new study area.

Erling Sögnen

Introduction

The rearing of various species of fish in fjords, rivers and lakes has been receiving increasing attention in the last decade. About 1000 tons of rainbow trout and salmon with a value of ca. 12 million kroner were sold from fish farms in 1971. It is estimated that in 15-20 years a production of 20,000 tons can be attained which will return 200 to 250 million kroner to the producers. As a comparison it can be mentioned that the landed value of the fish catch in this country today is ca. 1100 million kroner yearly. A successful fish-rearing industry would therefore yield a considerable income, especially to more remote areas of the country.

For the prognosis for fish rearing to be met would depend, for instance, on the ability to maintain good health in the rearing installations. Veterinarians will probably become involved in this work. It will be necessary to carry out research in many fields: anatomy, physiology,

virology, parasitology, pathology, etc. Fish pharmacology constitutes only a small, but still very important, part of the new multidisciplinary expertise which must be built up in this country. This article discusses anesthesia and pharmacological methodology since knowledge in these areas is necessary for further studies in this field.

Sedation and anesthesia

The rearing of fish involves tagging, weighing, measuring, stripping of roe and milt, and transportation. Laboratory experiments with surgical operations and blood and tissue sampling from fish are carried out as part of the research. The body shape of the fish and the p.314
slime layer means that the animals cannot be caught and held still by mechanical means without being severely stressed or directly damaged. Another difficulty is that they can be removed from the water only for a short time. Several specific requirements for sedation or anesthesia of fish have therefore appeared. In many cases it is definitely necessary to immobilize the fish in order to carry out the necessary manipulations, and the anesthesia will in any case make the work quicker, easier and less damaging for both the fish and those carrying out the operations.

Summary and definitions.

By studying the summary below listing the substances used to immobilize and calm fish, it can be seen that it is a somewhat heterogeneous group.

MS-222 - originally introduced (experimentally) as a local anesthetic, is now only used as an anesthetic for fish.

Quinaldine - not used in human or veterinary anesthesia, found after

systematic searching through a number of chemical substances in order to find a suitable fish anesthetic.

Methylpentynol - used as a sedative in human medicine.

Chloral hydrate - (hypnotic) used as basal anesthetic in veterinary medicine.

Tertiary amyl-alcohol - used as a hypnotic in human medicine.

Chloretone - used as a mild sedative in veterinary medicine.

Tribromomethanol - used as a general anesthetic for small animals.

2-Phenoxyethanol - used as a surface antiseptic in human medicine. Its effect on fish discovered accidentally when treating infectious diseases in fish.

Ether - an anesthetic in veterinary medicine.

CO_2 - the effect discovered during studies of the effect of various CO_2 concentrations in the water on fish.

The references in the literature are not very precise in their wording with regard to the classification of the substances, and the differences between sedatives, hypnotics and anesthetics are not clearly described. The expressions "anesthesia" and "anesthetics" are often used as a type of collective description. In this article the substances are divided into two large groups, namely sedatives and anesthetics, according to their effect on fish.and independent of their position in human or veterinary medicine. The word sedatives means substances that with a certain dosage result in reduced activity and reduced reactions to outside stimuli. Anesthetics mean substances that with a certain dosage will cause loss of consciousness with loss of pain sensation, muscle tone and reflexes. Some of the substances can be placed

in both groups depending on the concentrations used. The distribution of the substances in the two groups will be discussed at the end of the article.

Areas of utilization for sedatives and anesthetics on fish.

It has already been mentioned in the introduction those purposes for which these substances are useful in studies with fish. It is natural to divide the operations into two groups, namely:

1. Those requiring more or less deep anesthesia. Here we find tagging (marking), weighing, measuring, fin clipping, stripping of roe and milt, operations and sampling.
2. Those requiring only sedation. Transportation of fish is included in this group.

The results from a number of studies that have been carried out in this area are listed below. These are partly pure experiments to test new substances and partly experiments to try to solve practical problems encountered in laboratories and fish rearing stations.

1a) Weighing, measuring, tagging (marking), grading.

Eschmeyer (1953) reports on the use of ether anesthesia for fin clipping trout. In a trout rearing experiment, a large number of small trout were to be marked by fin clipping. After starting to immobilize the fish in a 1% ether solution, the number of fish marked per hour increased by ca. 28%. The work was generally carried out better after the ether bath was taken into use. The mortality following fin clipping was somewhat higher with ether than without, but the difference was not great enough to be of practical importance. Gerking (1949) used urethane solution for weighing, marking and measuring with good results. Urethane

has since been found to cause cancer when it comes in contact with skin and is therefore not of interest at the present time. Fish (1943) had problems in grading several types of fish after transportation in tank trucks. He developed a method for adding carbon dioxide to the tank. CO_2 in a concentration of 200 ppm was found to be an inexpensive and effective short-time anesthetic.

1b) Stripping roe and milt.

This operation results in a high mortality, according to Allison (1954). By using an ether bath, the mortality was lowered from 35.4% to 5.6%. After removing the fish from the ether bath, the fish were quickly rinsed in fresh water to prevent having ether mix with the roe or milt and damaging these. MS-222 has also been tried for this purpose. Allison (1961) measured the time of survival of trout sperm in clean water and in water containing varying concentrations of MS-222. It was found that MS-222 in a concentration equal to one-fourth of the amount necessary to anesthetize adult trout reduced the survival time of sperm to below 10 seconds.

1c) Surgical procedures and sampling.

Due to their scales, slime covered bodies, and their dependence on water, surgical procedures are not easy to carry out on fish. These have therefore not been carried out to any large extent. But some experiments have been reported, especially with salmonids. Several techniques are available, for instance blood sampling, insertion of vein catheters for repeated blood samples, removal of tissue samples and pumping stomach contents. These will be described in more detail in a separate article on experimental techniques. If the fish are to be kept

out of water for any length of time, the gills must be sprayed with an anesthetic solution.

2) Transportation.

Transportation of fish over longer distances and extended periods of time has only been carried out over the past ten years, but it is becoming more frequent due to the increase in fish rearing. The following factors can cause problems during transportation of fish:

1. Lack of oxygen.
2. Accumulation of waste products with the resultant changes in water quality.
3. Mechanical damage to the fish due to blows and shaking (vibration).
4. The fish becoming violently agitated.

The problem of lack of oxygen was recognized at an early stage. Attempts have been made to prevent this by supplying air, stirring arrangements, large volumes of water per fish, etc. It was found, however, that it was not only the oxygen content of the water that was of importance. The ability of the fish to utilize this oxygen is, for instance, affected by the amount of waste products in the water. Use of sedatives has made transportation of fish considerably easier, even if the problems are far from being solved. Under the influence of these substances the metabolism of the fish is reduced. They use less oxygen and excrete less waste products. There is less danger of injuries from blows since the fish are calm and passive. The fish do not become excited and use up all their energy by swimming nervously around in the container. High mortalities have occurred following transportation even when conditions have otherwise been good and this has been ascribed to excitement and overexertion.

Sedation is preferred to anesthesia during transport since it is more desirable to have the fish swimming around than lying on the bottom of the tank.

For more detailed information on transportation of fish publications by McFarland (1959) and Norris et al. (1959) are recommended.

Other references: (5, 7, 23)

Methods for giving sedatives and anesthetics to fish.

Sedation and anesthesia of fish are today carried out by adding chemicals. Many different methods were tried in the first experiments in this area, for instance electrical shocks or placing the fish in icewater or crushed ice. Such treatments put a heavy stress on the fish, and reliable results of experiments carried out under such conditions cannot be expected. It was therefore an important step forward when anesthetic substances were taken into use. The following methods of administering anesthetics have been tried: p.316

1. Intravascular injections.

   Since the circulatory system of the fish is located close to the body surface, intravascular injections are not of interest in this case. A method has been developed to go into the dorsal aorta of the fish for the purpose of taking blood samples and giving injections (see article on experimental techniques). But since a surgical operation of this type requires that the fish be already anesthetized, this method cannot be used for administering sedatives and anesthetics. Smith and Bell (1967) reported that they tried to administer MS-222 in this way but with poor results. All the fish died.

2. Intramuscularly and intraperitoneally.

This method is possible but has not been used very much. It would be most useful with a few large fish if it is not desirable to add the substance to the water.

3. Gill spraying.

The anesthetic is mixed with the required quantity of water where the fish are kept, and this mixture is sprayed over the gills. This requires that the fish are caught and that the head can be held firmly (see also comments under the chapter on MS-222).

4. Mixing sedatives and anesthetics in the water.

This is the method most frequently used. There is a certain disagreement over whether the substances should be added to the vessel where the fish are kept or if the fish should be transferred to a ready-mixed solution. McFarland and Klontz (1969) recommend, without further explanation, adding the active substances to the fish container while Klontz and Smith (1968) recommend that the fish be transferred into a ready-mixed solution. This is to avoid disturbing the fish during mixing. The vessel used must be of a material that will not undergo chemical changes, such as glass, plastic or stainless steel. It is furthermore very important that the fish have water of the same temperature and same chemical composition as they are used to. The fish should be starved 24-48 hours in advance and preferably also 24-48 hours after waking up.

Stages of anesthesia.

Articles with detailed descriptions on the use of sedatives and anesthetics on fish started to appear in the beginning of the 1960's. The substances had then already been in use for some years, but anesthesia was only considered to be a part of the experiments being described and not too many words were used to describe the procedure. These were therefore rather arbitrary, and the results were difficult to predict.

A summary of the behaviour- and reaction patterns in various stages of anesthesia has been assembled. The experiments carried out indicate that, with few exceptions, the same stages will occur with different types of anesthetics and also on various species of fish. It should be noted that such a behavioural summary is not the best way to evaluate stages of anesthesia. Exact data on the reaction of the fish organism would be more valid, but so far these have not been measured.

The summary has not been set up for a certain anesthetic used on a certain fish species, but is only meant as a rough guide.

In the first seconds the fish will often swim restlessly about due to irritation from the solution or possibly due to the transfer to a new vessel. The various stages can then be followed. There is a certain amount of controversy with regard to gill movements, but when the fish has reached stage II, 2 and III, the respiration will always be rapid and shallow and the movement of the gills is difficult to observe. An overdose manifests itself by a complete stop of movement of the gills, followed by overextension. The gills stand out stiffly in cramps at 15-20 second intervals. Even if the fish has been too deeply anesthetized, it can be rescued if it is placed in clean water (of the same type as it

is usually kept in). If gill movements have not started in ca. 1 minute, the fish can be slowly moved back and forth in a lengthwise direction.

Table 1. Classification of behavioural changes occurring in fish under anesthesia. p.317

Anesthesia stage		*Fish behaviour*
Stage Plan		
0	Normal	Reacts to outside stimuli, muscle tone and swimming ability normal.
I - 1	Light sedation	Decreased reaction to outside stimuli (light, touch)
I - 2	Deep sedation	Cessation of reactions to outside stimuli, except for heavy pressure, somewhat lower frequency in gill movements.
II - 1	Part loss of swimming ability	Reacts only to very strong stimuli (pressure and vibration). Part loss of muscletone. The fish is able to stay afloat, but swimming ability strongly reduced. Increased frequency of gill movements.
II - 2	Complete loss of swimming ability	Cessation of muscle tone, reacts only to deep pressure stimuli, frequency-of gill movements below normal.
III	Loss of reflexes	All reactions ceased, respiration and heart frequency lowered.
IV	Collapse	Gill movements ceased. Followed by heart stoppage after a few minutes.

Table is taken from McFarland (1959).

A solution will lose its effectiveness when it has been used for a number of fish. This can be detected by the longer time needed to put the fish to sleep and the difficulty in keeping deep anesthesia. Only dissolved and diluted anesthetic should then be added, otherwise poor mixing and death due to high concentrations in certain areas of the tank can occur. The solution should preferably be replaced since waste products would have accumulated in the used solution.

Choice of anesthetic.

In the choice of substances for sedation and anesthesia of fish, some of the same considerations as when treating mammals must be followed, but some new difficulties peculiar to fish will be encountered. A few factors that must be considered are listed below:

1. The fish species being handled.
2. The number of fish to be treated.
3. The length of time the sedation is to be maintained.
4. The treatment to be carried out.
5. The temperature of the water.
6. The chemical composition of the water.
7. The toxicity of the substance.
8. The price.

For most of these points there is, unfortunately, quite limited knowledge and experience available. Some information is available in the literature for some specific problems, but it is largely still necessary to use trial and error.

Information on individual substances.

While reading or using the following summary, one should note that the dosages and times given are only guidelines or a kind of average value for the fish species the substances have been tried on. It is assumed that there is little interest in having the exact dosages of a certain fish species given under certain conditions, since these cannot be directly applied to another species and other conditions. The information on the effect mechanism of these substances on fish has not been included in the summary since there appears to be few data available in this area. For most of the substances it has not been possible to find data on therapeutic indices on fish or other data on toxicity except LD50 for mice. p.318

MS-222

General characteristics: MS-222 (Metacaine, m-aminobenzoic acid ethyl ester methane-sulphonate, $C_{10}H_{15}NO_5S$, is a white, crystalline powder.

Solubility: 1 g/0.8 ml water at 20°C. Gives a clear, colorless solution with a slightly acid reaction.

Stability: Crystalline powder is stable if it is kept dry and cool. A 10% solution which was stored at room temperature showed no loss of activity in 3 days, but after 10 days the effect was reduced slightly. The stability of the solution decreases with increasing storage temperature. Light does not appear to have any adverse effect, but a color change to yellow or brown can occur (Bové, 1962).

Dosage: MS-222 has been reported to be used in concentrations from 50 mg/l water to 1 g/l water depending on fish species, water quality, desired effect, etc. Specific references must be consulted and each case tried on its own.

Effective time: The times for the drug to take effect: 15 sec-4 min. The fish can be held in the solution for a relatively long time without ill effects, according to Gossington (1957) up to 2 days. Gossington does not indicate, however, if in his experiments sedation or surgical anesthesia were involved. Recovery time: 3-15 min.

Areas of utilization: MS-222 is recommended as the best compound to attain surgical anesthesia and it is also recommended for areas such as marking, fin clipping, roe stripping and studies on live fish, but it is then used in lower concentrations.

Toxicity: According to Bell (1964), MS-222 is three times less toxic than Novocaine and ten times less toxic than cocaine. It can have toxic effects on the fish if used in water colder than that normally occupied by the fish.

Notes: Certain warnings against the use of MS-222 have been published, as pointed out earlier. Bell (1964) claimed that a concentration of 19 ppm will damage trout sperm. McFarland and Klontz (1969) warned against carrying out the experiments in sunshine. Toxic compounds can then be formed. Certain trout species are hypersensitive to MS-222. An advantage of the compound is that fish that have been treated can be eaten without ill effects. This opens possibilities for easier catching of large fish species. Gilbert and Wood claim, according to Sandoz publication No. 3, 1962, that they can spray a MS-222 solution over the gills of sharks by placing a special type of waterpistol in their mouths. The fish are then anesthetized in a short time and can easily be killed.

Availability: Can be ordered through pharmacies.

Other references: (2, 5, 7, 12, 22, 23).

Quinaldine

General characteristics: Quinaldine (2-methyl quinoline, $C_{10}H_9N$) is a colorless, oily liquid. Not very flammable.

Solubility: Very low solubility in water. Acetone is recommended as a solvent, since acetone does not affect or damage the fish. Ether, ethanol and chloroform are also used as solvents.

Stability: The undiluted liquid becomes reddish-brown if it is exposed to air, and containers must be kept tightly closed. Solutions retain their effect after several days' storage.

Dosage: The recommended dosages vary somewhat. They are in the area of 0.005-0.03 ml quinaldine in the same quantities of acetone per liter of water, depending on fish species and desired effect.

Effective time: The immobilization time is given as 1-3 min, with outer limits of 45 sec and 6 min depending on the concentrations and the fish species used. Sedation can be maintained for relatively long times without ill effects. Muench (1958) describes an experiment where green sunfish were held in 0.005 ml quinaldine per liter of water for 48 hours and yellow bullhead was held in 0.007 ml quinaldine per liter of water for 70 hours. Several weeks of observation showed no damage. Most species can tolerate, for short periods, a dosage three times higher than necessary for complete loss of equilibrium. The recovery time is given as 1-20 min, depending on concentration and time of exposure. p.319

Areas of utilization: Quinaldine can be used over quite wide temperature ranges (13-26°C). It promotes good muscle relaxation. Bell (1964) recommends quinaldine for blood sampling and regular handling. The

substance has not been extensively used for surgical procedures. It should, according to Bell (1964) give surgical anesthesia to certain species.

Toxicity: Preliminary experiments show low toxicity. LD50 (oral) for mice: 1.23 g/kg. Lengthy inhalation should be avoided since quinaldine is strongly irritating to mucous membranes. Therapeutic index for fish is given as ca. 3 for the species where this value has been determined.

Availability: Can be obtained through Norwegian Medicinal Depot. Delivery time ca. 5 weeks.

Other references: (12, 13, 22).

Methylpentynol

General characteristics: Methylpentynol (meparphynol, $C_6H_{10}O$) is a volatile liquid with a sharp smell and burning taste.

Solubility: Solubility in water at 25°C = 12.8 g/100 ml. Soluble in ether. Mixable with for instance acetone, benzene and carbon tetrachloride.

Stability: Stable non-diluted and in solution.

Dosage: 0.5-0.9 ml/l water.

Effective time: According to McFarland and Klontz (1969), the effect will occur in 2-3 min and the recovery in 5-20 min.

Areas of utilization: Difficult to maintain a deep anesthesia due to respiratory difficulties. It will not give good muscle relaxation. Recommended as a sedative for transportation.

Toxicity: LD50 orally for mice = 525 mg/kg.

Comments: If air is bubbled through the solution, an antifoam compound must be added.

Availability: Can be obtained through Norwegian Medicinal Depot. Delivery time ca. 5 weeks.

Other references: (3, 12, 22).

Tertiary Amyl Alcohol

General characteristics: Tertiary amyl alcohol (2-methyl-2-butanol, $(CH_3)_2$-C(OH)CH_2-CH_3) is a volatile liquid with characteristic smell and burning taste. Must be kept in closed containers protected against light.

Solubility: Soluble in 8 parts water, mixable with alcohol, ether, benzene, chloroform, glycerol.

Stability: Stable both undiluted and in solution.

Dosage: 1-1.3 ml/l water in order to obtain complete loss of equilibrium. 1.6-1.8 ml/l water for complete loss of reflexes (McFarland, 1960). 0.5-1.25 ml/l water for transportation (McFarland and Klontz, 1969).

Effective times: Takes effect in 10-20 min. McFarland (1960) reports that Girella (tropical saltwater fish) could be kept in a solution with a concentration of 1 ml/l water for 3 hours without ill effects. The recovery time was 20 min. *Thunnus germo*, a tuna species, was placed in solutions with concentrations up to 3.1 ml/l. Loss of reflexes occurred after 1-3 min. The fish were apparently undamaged after the treatment. Recovery time for this experiment was not reported. McFarland and Klontz (1969) indicate that the recovery time is 20-90 min.

Areas of utilization: Recommended for minor manipulations, marking, ordinary handling and transportation.

Toxicity: The compound is moderately irritating on the mucous membranes in humans and also anesthetic in high concentrations.

Comments: If air is bubbled through the solution, an antifoam compound must be added.

The fish are excited the first 15-20 sec after having been transferred to the solution.

Availability: Can be ordered through pharmacies.

Other references: (3, 22).

Chloral Hydrate

General characteristics: Chloral hydrate ($C_2H_3Cl_3O_2$) occurs as colorless, transparent crystals with an aromatic, penetrating odor.

Solubility: Chloral hydrate is easily soluble in water, 4.9g/ml water at 15°C, 8.3 g/ml water at 25°C. Soluble also in alcohol, chloroform, ether. p.320

Stability: The solid is volatile when exposed to air. The diluted solution is relatively stable if it is kept cool and protected against light.

Dosage: 0.8-0.9 g/l water.

Effective times: The effect is noted after 2-10 min. Recovery requires 20-30 min. It is difficult to obtain deep anesthesia.

Areas of utilization: Chloral hydrate has mainly been used for transport of fish where long-lasting sedation is desirable.

Toxicity: LD50 orally for rats: 500 mg/kg. Chloral hydrate is a local irritant. Can produce gastrointestinal disturbances and affect respiration and heart rate in humans.

Other references: (3, 13, 22).

2-Phenoxyethanol

General characteristics: 2-Phenoxyethanol ($C_8H_{10}O_2$) is an oily liquid with mildly aromatic odor and burning taste.

Solubility: Solubility in water = 2.67 g/100 ml. Freely soluble in alcohol and ether. Bell (1964) recommends making a stock solution with alcohol or warm water.

Stability: 2-Phenoxyethanol is stable both undiluted and in solution.

Dosage: The following information is taken from an article on anesthesia of salmon (Sehdev, McBride and Fagerlund, 1963). The fish were kept in normal water at 11°C.

ED50 at 11°C = 0.1 ml/l water

ED50 at 4°C = 0.06 ml/l water.

The desirable effect is obtained when fish are not reacting to light or pressure.

Areas of utilization: For marking and general handling. Combined with low water temperature for transportation.

Toxicity: 2-Phenoxyethanol must not be drunk. Will stimulate, later depress the central nervous system, vomiting, respiratory difficulties, coma.

LD50 for fish at 11°C = 0.3-0.4 ml/l water. Therapeutic index >3.

LD50 for fish at 4°C = 0.3 ml/l water. Therapeutic index = 5.

Other references: (12, 13, 22).

Chloretone

General characteristics: Chloretone (chlorobutanol, $C_4H_7Cl_3O$) occurs as crystals with camphor odor and taste. Will sublime easily.

Solubility: Low solubility in cold water. Easily soluble in warm water, chloroform, ether, alcohol, acetone. If chloretone is to be used in the field, it is recommended that a stock solution be made in advance if only cold water is available there.

Stability: A 10% stock solution made with warm water will be stable at 4°C for a long time.

Dosage: Reported to be as different as 8-10 mg/l water (McFarland and Klontz, 1969) and 200-400 mg/l water at 3-10°C (Bell, 1964).

Effective times: Effective in 1-3 min. It is not indicated if these dosages will give sedation or anesthesia, or what the maximal effective time would be. Recovery requires 2-30 min.

Areas of utilization: Chloretone in these dosages is recommended for short-time anesthesia for marking, measuring, weighing, etc.

Toxicity: MDL orally for dog: 238 mg/kg.

Other references: (6, 17, 22).

Tribromoethanol

General characteristics: Tribromoethanol (Br_3CCH_2OH) occurs as crystals with a faint aromatic odor and taste.

Solubility: Soluble in 40 parts water at 40°C, also soluble in alcohol, ether, benzene.

Stability: Aqueous solutions decompose when exposed to light and dibromo-acetaldehyde and hydrogen bromide are formed. Both compounds are strongly irritating for fish and humans. Marketed under the name Avertin with amylene hydrate added to form a stable solution.

Dosage: 5-50 mg/l water.

Effective times: Effective in 5-10 min, recovery in 20-30 min.

Areas of utilization: Tribromoethanol has been used for short-time experiments.

Toxicity: LD50 orally for cats: 150 mg/kg.

Other references: (3, 13, 22).

Ether

General characteristics: Ether ($C_2H_5OC_2H_5$) is a colorless, volatile and very flammable liquid. Characteristic sweet, sharp smell and burning taste. p.321

Solubility: Ether is soluble in water, saturation at 8.43% w/w at 15°C.

Stability: Undiluted ether is stable if it is not exposed to sun, air and humidity.

Dosage: 10-15 ml/l water.

Effective times: Effective in 2-3 min, recovery requires 5-30 min. McFarland and Klontz state that the effect can be maintained over moderate periods of time.

Areas of utilization: Ether in these dosages has been used for instance for fin clipping and stripping of roe and milt.

Comments: Ether is strongly irritating in a concentration of 40-50 ml/l water.

Other references: (1, 3, 8, 22).

Carbon Dioxide

General characteristics: Carbon dioxide (CO_2) is a colorless, odorless, non-flammable gas.

Solubility: 88 ml CO_2/100 ml water at 20°C, 760 mm Hg.

Dosage: 200 ml/l to obtain complete loss of equilibrium and immobilization of mature trout and salmon (Finch, 1943).

Effective times: While CO_2 is being added to the water, the fish will swim around restlessly. 1-2 min after full concentration has been reached there is loss of equilibrium and the fish sinks to the bottom. Fish should be removed from the solution after 5 min. After having been transferred to clean water, they will be in full activity and without visible damage in 5-10 min.

Areas of utilization: The method was used by Finch (1943) for separating mature salmon and trout that had been kept in a common tank. Finch also recommends dipping fish in CO_2 solution before stripping roe.

Addition of CO_2: The desired concentration is obtained by adding dissolved sodium bicarbonate ($NaHCO_3$) and diluted sulphuric acid. The quantity of each compound is calculated according to the molecular weight. The concentration can be regulated by adding sodium carbonate, which neutralizes CO_2.

Comments: The method is inexpensive, but requires great accuracy and vigilance. It does not seem to have attained any popularity and is not often used. It is mentioned because this was one of the first experiments carried out on anesthesia of fish.

Other references: (3, 13, 22).

Urethane

Considered to be a safe and good anesthetic for fish. Gives rapid effect which is easy to maintain, and in addition, rapid recovery. Urethane was used extensively until it was found to be carcinogenic in contact with skin. The chemical is no longer in use for fish anesthesia.

References: (10, 13).

Short summary of the utilization of these substances.

The substances are arranged in the summary according to how important and how utilized they are in studies on sedation and anesthesia of fish. It was mentioned in the introduction that two types of problems can be listed, namely:

1. Working problems requiring that the fish are caught and taken out of the water. Anesthesia is required for this. Most authors recommend MS-222, Quinaldine or tertiary amyl alcohol.
2. Transportation where only sedation is required. For this, chloral hydrate, methyl pentynol and tertiary amyl alcohol are recommended.

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12b. Food fish rearing - a new industry. Norges Vel, 1972, 6, 2-12.

Experimental methods

The basic principles for surgical operations on fish. p.323

The discovery of substances to keep fish in surgical anesthesia up to several hours opened up many possibilities for surgical operations on fish. The earliest experiments are incompletely described, but in 1967 Smith and Bell published a description of anesthesia and surgical operations on Pacific salmon. Their work forms the basis for later experiments on fish surgery and experimental techniques.

1. *Anesthetic equipment.*

In the first surgical experiments that were carried out on fish, the fish was lying on its side with the head in water containing anesthetic or one of the gills was sprayed with anesthetic solution. None of these procedures proved to be ideal. Smith and Bell liked to have the fish lying on its back. This gave them two advantages, namely: 1) It was easy to get into mouth and stomach cavities, and 2) both gills could be sprayed at the same time. They constructed a type of anesthetic apparatus that made it possible to obtain a reliable anesthetic state and good working conditions during the operation.

From the sketch, it can be seen that there are possibilities for adding the anesthetic solution either from the outside or by mouth. In both cases the solution passes through a spreading in order to damage the gills as little as possible. It did not appear to be of any importance for the gills, the revival time, or the survival rate, as to the direction in which the water was pumped.

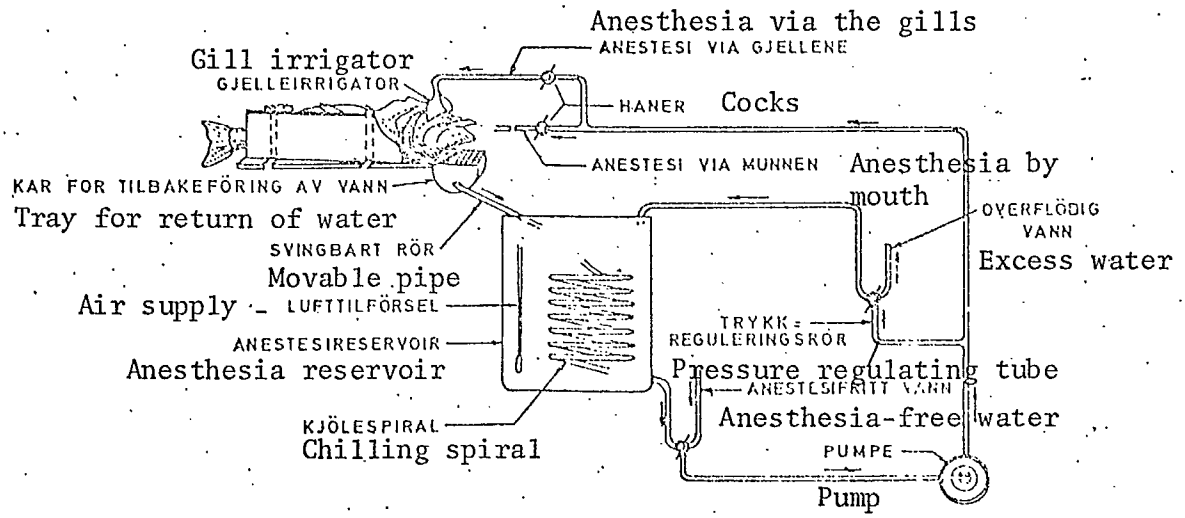
The anesthetic solution, in this case MS-222 in a concentration of 100 mg/l for fish from 200 g to 4000 g, was chilled before it was pumped

over the gills. An extra effect from the chilling was then obtained and somewhat lower concentrations could be used. The solution was also oxygenated.

2. *Other equipment.*

A V-formed trough, which is lined on the inside with a soft water-insoluble material, is needed. The fish is placed in this trough. In order for the fish to be immobile, a hook can be fastened to the skin near the anal fin and tied to the vessel. For the same purpose, a couple of soft rubber bands can be stretched over the fish. In order to keep the mouth open a hook can be fastened in the lower jaw. Furthermore, a damp cloth is needed to cover the fish, an aspirator to remove excess water, physiological saline and necessary surgical instruments.

Fig. 1. Schematic drawing of an anesthesia apparatus for surgery (Smith and Bell, 1967).



Several types of physiological saline solutions have been tried. Cortland saline has proven to be most suitable. If more than 10% of the blood volume in a salmon is replaced with 1% NaCl, the fish becomes restless and shivering. However, if the Cortland solution is added in such quantities that the hematocrit value drops from 31% to 3%, these reactions cannot be observed (Smith and Bell, 1967). p.324

Fig. 2. Operating table and anesthetic equipment.

A - anesthetic solution

B - reservoir for untreated water

C - circulating pump

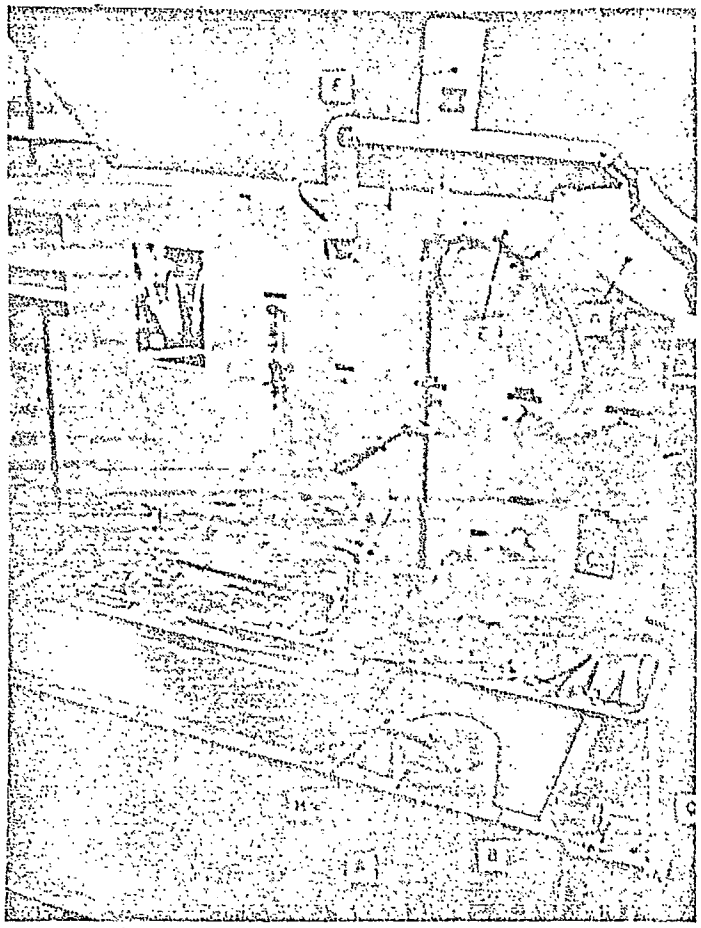
D - gill-irrigation equipment

E - mouthpiece for oral addition of anesthetic solution

F - salt solution

G - light

H - movable tube for returning water or anesthetic solution (Klontz and Smith, 1968)



3. *Procedure.*

After having been without food for 48-72 hours, the fish is first anesthetized in a vessel with the same anesthetic as later used in the gill irrigation system. It is placed in the trough and the apparatus connected to gills or mouth. The fish can, if necessary, be fastened down. The area to be treated is carefully wiped clean of slime with sterile tampons. The slime is considered to have antibacterial properties so further treatment is unnecessary. The required manipulations can now be carried out. The fish is lying calmly with a minimal possibility for being mechanically damaged. The level of anesthesia can be regulated by alternating between clean water and anesthetic solution, and when the experiment is completed the fish is released in clean water. It should not be fed for the next 24-48 hours.

Taking of blood samples.

In the first trials with taking blood samples the fish had to be sacrificed. In the case of small fish, the blood sample was taken by cutting a gillraker. In larger fish the heart or one of the larger blood vessels was punctured. Heart puncturing in fish is carried out in the same way as in mammals. The anesthetized fish is placed on its back. A glass syringe and a sharp needle is used. The point of penetration is in the crossover point between linea alba and an imaginary line between the cranial points of attachment of the pectoral fins (salmonids). p.325

However, there can also be requirements for repeated samplings from the same fish. R.H. Schiffman (1958) describes a method for blood sampling from the dorsal aorta via the mouth.

Level with the fourth gillraker, in the middle of the roof of the mouth, the eight gill veins are joined to the dorsal aorta.

The point of penetration is therefore just behind the fourth gillraker. Needle No. 22 and a glass syringe were used for a 1200-gram trout. The anesthetized fish (MS-222 used in the original experiments) is placed on its back and the needle is inserted so that it forms an angle of 45° with the roof of the mouth. After the fish has been anesthetized, a skilled technician should be able to take a blood sample in 15-30 sec.

The method is limited to fish with large mouths. In a fish with a very small mouth it should be possible to reach the dorsal aorta via the gill openings, or by inserting the needle level with the first gillraker and pushing backwards until the vein is reached. These procedures are, however, insufficiently described.

Blood sampling through the dorsal aorta can now be done much more elegantly. Smith and Bell published an improved version of Schiffman's method in 1964. Mature salmon were used. The fish was anesthetized, placed on its back and the gills irrigated with MS-222. The mouth was opened and a hole made in the middle line just in front of the nostrils with a sharp needle. A short piece of plastic tubing was placed in the hole and was allowed to protrude 5-10 mm past the bridge of the nose. A new piece of plastic tubing somewhat thinner and longer than the first was fastened to a sharp needle. This was placed in the middle of the roof of the mouth level with the first gillraker. The thin plastic tubing was pulled out through the nose opening and fastened. The needle could now be pushed further in until the aorta was penetrated, which was demonstrated by blood running out and filling the tube. The tube could be plugged with a glass stopper. The needle-tubing system was, during the experiment, filled with heparinized physiological saline.

Fig. 3. Point of injection and correct needle angle shown on whole fish and fish where the lower jaw has been removed surgically (Schiffman, 1958).

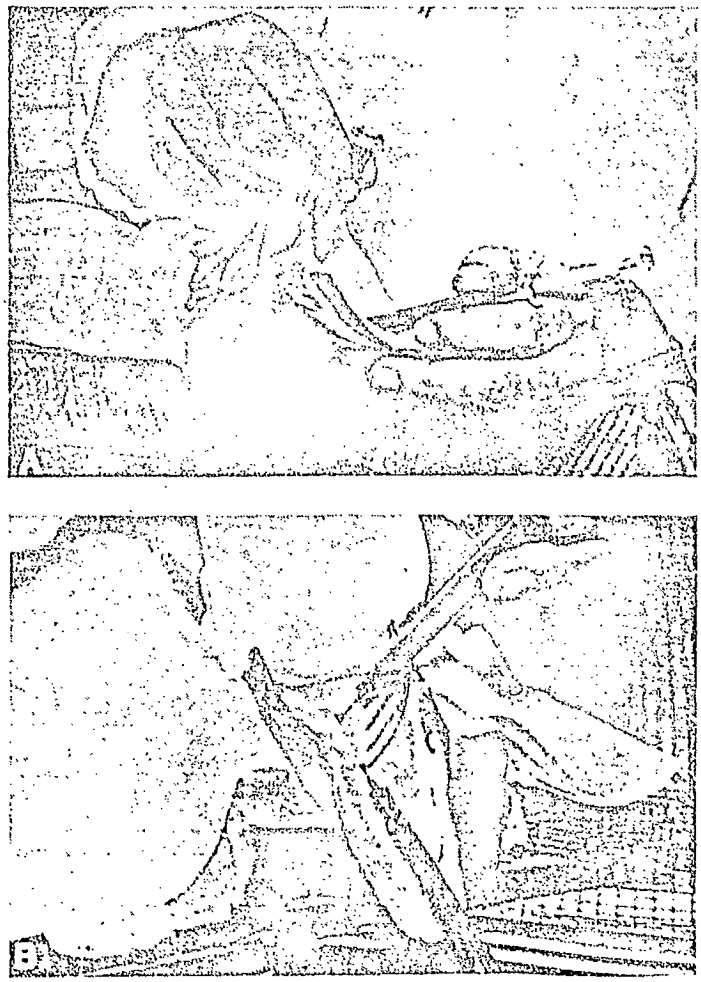


Fig. 4. Schematic drawing of gillrakers and blood vessels (Schiffman, 1958).

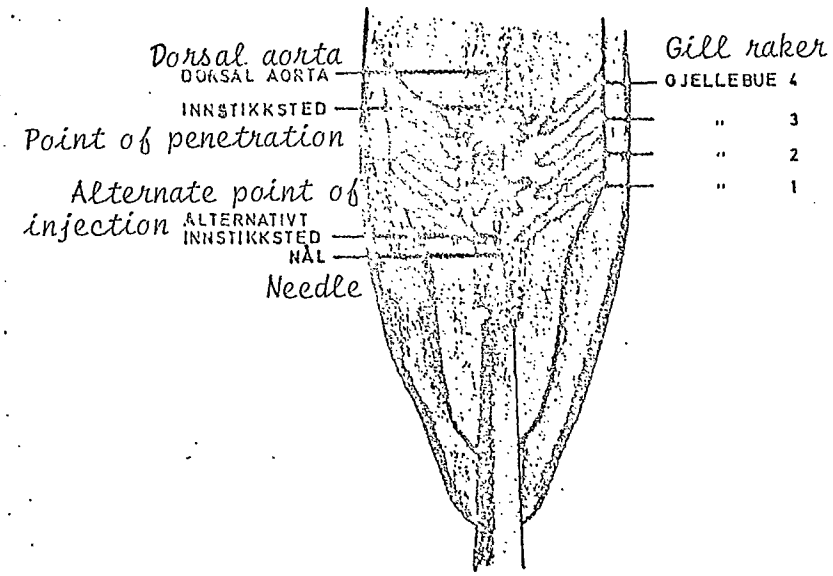


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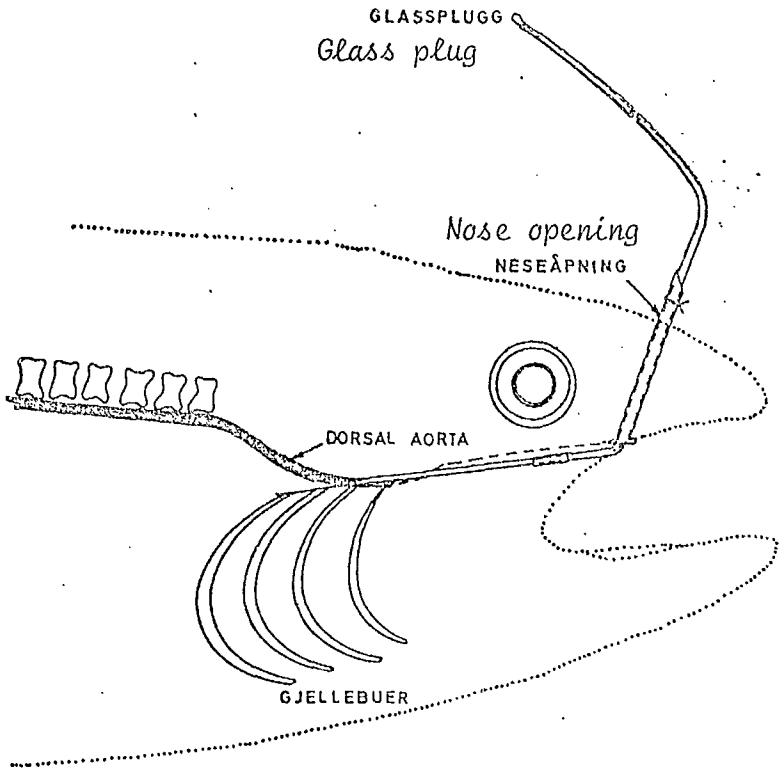
Fig. 5. Sagittal section of salmon with needle inserted (Smith & Bell, 1964).



The fish now had the gills irrigated with clean water until normal respiratory movements resumed and was then moved to a small aquarium. It was left there for 4-6 hours until all the effect of the anesthetic had disappeared. When a blood sample of the fish was to be taken, it was captured by grasping the plastic tubing with long pliers. The end of the tubing was held over water, the glass stopper removed, saline and the first drops of blood gathered and discarded, and the blood sample then taken. The blood was replaced with saline and the tube filled up again. p.327

This method has a great advantage in that blood samples can be taken under approximately normal conditions for the fish. The fish does not appear to be bothered by having the plastic tubing through the nose, and does not appear to be frightened when it is towed in by this tube. In tables on blood values for fish, the conditions under which the samples are taken should always be exactly specified since this has a considerable influence on the values obtained. Blood samples from a fish that has been caught, anesthetized and placed on its back must not be compared with samples from a free-swimming fish.

The method can also be used, with few modifications, for repeated intraarterial injections (Smith and Bell, 1964).

We are warned against drawing too large a sample of blood. The amount that can be removed in a sample without damaging the fish varies from fish to fish. For salmonids, about 1% of the body weight which corresponds to 25-30% of the blood volume, could be withdrawn. With large withdrawals there are also difficulties with the increased tendency to coagulate. Fish blood has always been considered to coagulate very rapidly, but when taking blood samples directly from the aorta,

under minimal stress and with small withdrawals, the coagulation does not present a problem (Smith and Bell, 1967). Recommended anticoagulants are heparin, sodium citrate, sodium oxalate and EDTA.

Attempts have also been made to go into the other large arteries in the body. These techniques are, however, so new and untried that there is no point in describing them in a review article. We refer to the references.

Measurement of excretion products.

In many cases it is necessary to collect urine or excretion products from intestine or gills in order to measure metabolites after administering toxic substances, antibiotics, sulphur, anesthetics, etc. An isolation chamber that makes such collections possible will be described here. The chamber was constructed by Post, Shanks and Smith in 1965.

In order to collect urine, a urine catheter must first be inserted. The method for catherizing fish must be adjusted for each species since there can be quite large anatomical variations. In salmonids, which are most often used for experiments, the catherization should be a relatively simple operation. A polyethylene tube is inserted in the anus and is carefully moved until it enters the urethra. The tube is carefully pushed forward until it reaches a constriction. It should barely pass this constriction. If the tube is pushed further in, the kidneys might be damaged. The catheter is fastened on the outside with a suture to the anal fin or to the skin between the anal fin and the anal opening (Klontz and Smith, 1968).

With the catheter inserted the fish can be moved to the isolation chamber.

The chamber is divided into front and rear compartments with the aid of a rubber belt fastened to the fish and to the vessel, as shown in the sketch. In the first experiments the belt consisted of the upper part of a rubber glove.

With this arrangement it is possible to measure: 1) Quantities of urine and its composition. 2) Excretion products from intestine by analyzing the water in the rear compartment. 3) Excretion products from the gills by analyzing the water in the forward compartment. This water must then be circulated, purified and oxygenated.

Taking tissue samples.

Tissue samples can be necessary both for histological and chemical studies. An ordinary biopsy needle can be used also for fish, but larger quantities than those that can be removed with a biopsy needle are necessary for chemical studies of tissues. A special instrument has been constructed for this purpose (Newman, 1963). The instrument consists p.328 of an outer and inner cannula. The outer is designed to cut through the muscle tissue. The inner part is at one end equipped with four sharp teeth designed to cut loose a piece of tissue. When in use, the needles are placed one inside the other. The teeth of the inner cannula are separated by a washer. The needles are inserted into the tissues, the washer removed and the teeth will cut a piece of tissue loose and pull it out.

Pumping of stomach content.

If the stomach content of a fish is to be analyzed, this can, of course, be done by sacrificing and cutting up the fish. But there can also be requirements for studying the same fish several times. Seaburg

(1957) describes a method for pumping stomach content. Two copper pipes are placed on top of each other and soldered together. One should be quite thin and the other of a size so that the two pipes can be inserted into the oesophagus of the fish. A rubber tube with a rubber bulb in the middle is fastened to the thin pipe. By pumping water down through the thin pipe the stomach content will be forced out through the thick pipe. Larger particles that may get caught and plug the tube can be taken up separately.

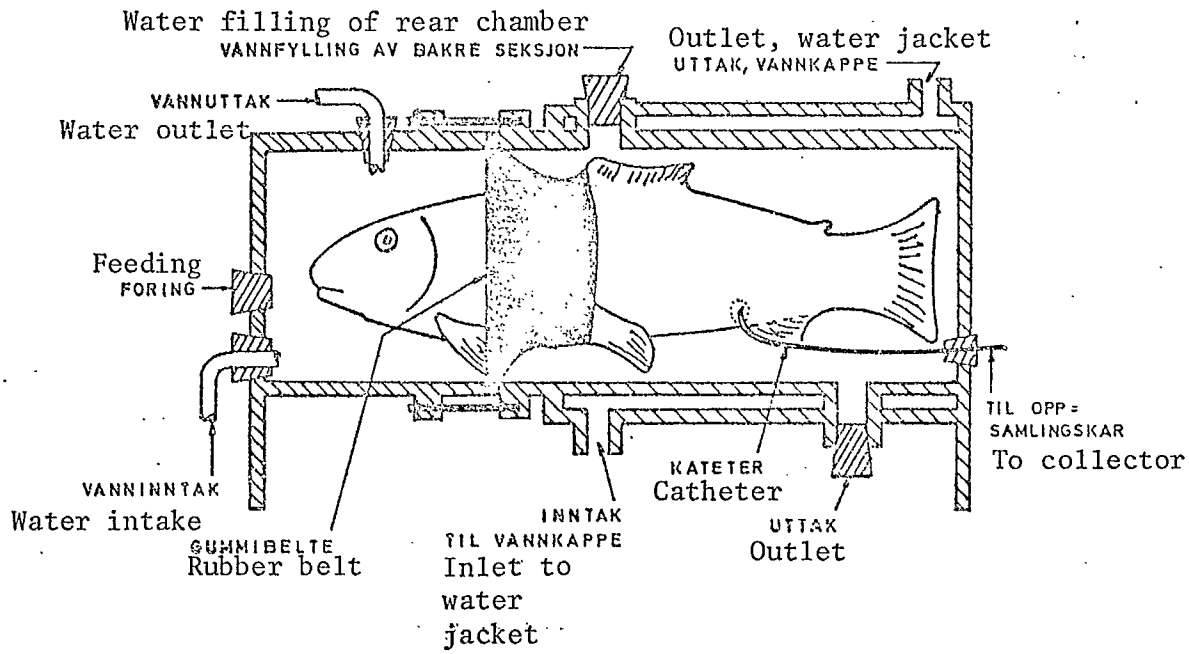


Fig. 6. Cut through a metabolism chamber (Post, Shanks and Smith, 1965).

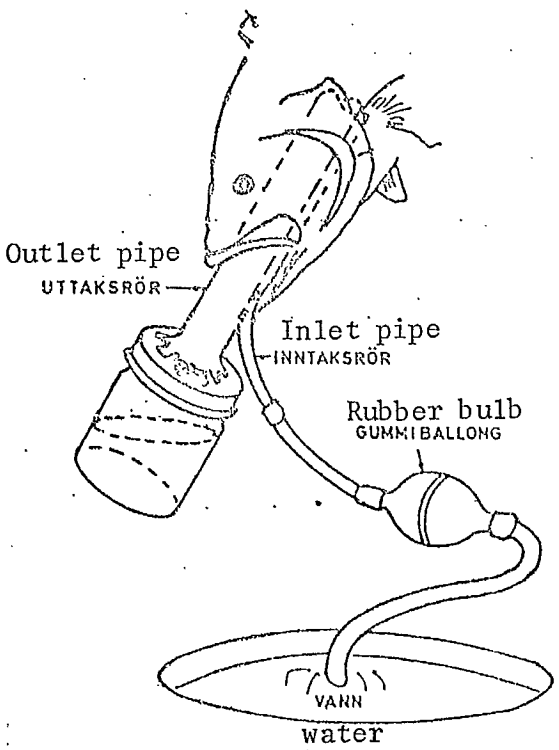


Fig. 7. Fish with stomach pump in place (Seaburg, 1957).

Force-feeding with test substances.

In the original experiment (Nahatani, 1962) fish were force- p.329
fed with radioisotopes, but the procedure could be used for all test substances required to be given in certain quantities. The method is really quite simple. The substance to be given is encapsuled in gelatin. A "capsule-box" is used which consists of an outer cylinder with an inner rod that can be withdrawn with a spring. The fish is anesthetized before feeding, the gelatin capsule placed in the box which is inserted past the throat of the fish where the capsule is liberated. The fish should be under observation for the first 3-4 min since the capsule can be regurgitated soon after administration.

As mentioned in the introduction, the purpose has been to refer to experimental techniques that can possibly be used in fish pharmacology studies in the future. Studies on the periphery include measurement of blood volume (ref. 1, 3, 9, 14), blood pressure (ref. 8) and blood flow (ref. 2).

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