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Ethyl Urethane as a Narcotic for Aquatic Animals.

By

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Ethyl urethane, $\text{NH}_2\text{-CO-OC}_2\text{H}_5$, carbaminic acid combined with ethyl alcohol, was introduced as a narcotic by Schmiedeberg in 1886¹⁾. It has since been employed a good deal for narcotic purposes on mammals, especially rabbits, being dissolved in a little water and introduced into the stomach in doses of about 1 g per kg. Schmiedeberg tested it on frogs and states that doses of 20 to 30 mg would diminish the voluntary movements, while 40 to 60 mg would suspend all movements both voluntary and reflex. He says: „Man könnte das Tier in diesem Zustande für tot halten, wenn nicht die kräftigen Herzschläge das Fortbestehen des Lebens genügend darthäten. Nach 36 bis 48 Stunden tritt auch aus diesem Zustande gewöhnlich vollständige Erholung ein, ja es ist kaum möglich, einen Frosch durch das Urethan zu töten, ohne ihn förmlich mit der Substanz einzubalsamieren“.

Öhrwall²⁾ tested Schmiedeberg's statements, but found that he must have meant cg instead of mg. For the microscopic study of the circulation in the lung of the frog Öhrwall employed a 25% solution of urethane in saline which he injected subcutaneously (1 cc for a frog of 50 g, and he describes the effect as excellent. The circulation is not at all interfered with and the narcosis will last for about 24 hours.

¹⁾ Archiv f. experim. Pathologie u. Pharmakologie, **20**, S. 207.

²⁾ Skand. Arch. Physiol. 1911, **25**, 11.

Following Öhrwall I have used the substance for a very large number of experiments and demonstrations on frogs, but I soon gave up the injections and simply poured a little 5% urethane solution over the frog in a beaker. After a few minutes the narcosis sets in without any preliminary excitation stage, and when it is sufficiently complete for the purpose of the experiment in view the frog is taken up, and the surplus of the solution is washed off with water. I find that when the drug is applied in this way, and when the frog is kept in a moist atmosphere, but without coming in contact with water, it will remain narcotized for any length of time up to at least 9 days, while complete recovery can be brought about in an hour, when desired, simply by putting the animal into cold running water.

When a frog is kept deeply narcotized for a long time it must depend for its respiratory exchange on the cutaneous respiration, which is sufficient only at low temperatures. I found, however, that a certain stage of narcosis could be induced by which all voluntary movements except the respiration were extinguished, while the reflexes, though not absolutely absent, were reduced to a minimum. To bring about this state by means of a 5% solution of urethane was very difficult, however, and to a great extent a matter of chance, but if a frog is brought into a larger amount of a weak urethane solution, the drug will diffuse in slowly and a state of diffusion equilibrium will gradually develop. By adjusting the strength of the solution any desired degree of narcosis can therefore be induced and kept up indefinitely. A concentration of 0,43% will produce in a frog that stage at which only the respiratory movements are maintained.

I have found this latter form of ethyl urethane administration especially suitable in dealing with aquatic animals, which must be kept in the narcotizing solution (or dry) for the whole length of time during which it is intended to maintain the narcosis.

I have examined the effects of ethyl urethane on the following animals.

Amphibia:

The effects on adult frogs are described above. On a tadpole 0,37% urethane prevented spontaneous movements. The breathing was regular and the circulation good. The animal died after 6 hours. 0,48% extinguished the reflexes and had a visible effect on the respiratory movements. The animal recovered in water.

Fishes (*Cyprinus auratus*):

0,45% urethane in water produces in a couple of hours an all but complete narcosis with feeble respiratory movements at long intervals. The narcosis was kept up in one case for two days, and complete recovery was obtained in pure water after a few hours. The narcosis was used for experiments on the respiratory exchange, and it was found that the urethane did not in the least interfere with the oxygen titrations.

Insects.

Chironomus larvae were completely and rapidly narcotized in 1% urethane. After being brought into 0,37% they recovered partially. 0,48% induced again a complete narcosis which was kept up for 4 hours. After a few minutes in water there was a complete recovery.

Urethane cannot be used to narcotize all aquatic insects and probably only such as breathe dissolved oxygen as distinct from air breathers. I have tried it on *Dytiscus* larvae, but saw no effect whatever.

Crustaceans.

Asellus. 0,48% urethane produced a practically complete narcosis with very feeble movements of single legs. The respiratory movements of the branchiferous appendages were regular however. When transferred to water after 4 hours an Asellus recovered in 15 minutes.

Idotea. In 1% urethane in sea water a complete narcosis was produced, but only very slowly. The respiratory movements were distinct and the circulation good. A complete recovery was obtained in pure sea water.

Daphnia sp. 0,4 to 0,5% urethane produces an incomplete narcosis. The animals cannot maintain themselves in the water but sink to the bottom. 0,55%: movements of legs at long intervals, none of antennae. After 12 hours the heart beat is not visibly affected, and the animals recover completely, though somewhat slowly, when brought into pure water. 0,6 to 1% urethane immobilizes the daphniae completely. After 15 minutes in a 1% solution they recovered rapidly in water.

The daphniae react so quickly and their behaviour in solutions of urethane is so typical and constant that it is possible to judge the strength of such a solution with considerable accuracy by using

daphniae as indicators. This has been utilized in the experiments made on the crayfish.

Cyclops sp. requires a higher concentration of urethane (0,6 to 0,7%) to become narcotized and, though smaller, reacts much more slowly than the daphniae.

Astacus fluviatilis. In a solution of 1% urethane a crayfish was not visibly affected and even after 12 hours in a 2% solution it was not nearly narcotized, though visibly affected. It was then killed, the blood was collected and after coagulation and filtration tested by means of daphniae. It did not narcotize them completely, but by gradual addition of 1% urethane it was found that the urethane concentration of the blood was nearly 0,4%. *Astacus* blood which did not contain urethane was found to have no narcotic properties and to require the addition of an equal volume of 1% Urethane to "precipitate" the daphniae.

I thought that the explanation of the incomplete narcosis might be that the gills of the crayfish are practically impermeable to urethane, but that some of the solution might have got into the stomach and been absorbed there, and I made therefore the following experiments:

One crayfish (of 50 g weight) had 2 cc 25% urethane injected into the blood.

Into a second 1 cc of the same solution was injected.

In a third the stomach was repeatedly filled with 5% urethane and the animal was kept in a moist atmosphere but not in water to see that the fluid was retained.

In a fourth a glass stopper was inserted in the mouth and tied firmly to prevent any fluid from getting into the stomach. The animal was then kept for 12 hours in a 2% solution of urethane.

No. 1 became very deeply narcotized and the respiration ceased completely. It was dead after 10 hours, and the blood was found to contain between 0,9 and 1% urethane.

No. 2 was narcotized in so far as it made practically no spontaneous movements beyond the respiration. The reflexes were not completely extinguished however. It recovered completely after 12 hours in running tap water.

No. 3 became deeply narcotized and died.

No. 4 was very slightly affected or perhaps not at all, as it very probably suffered at the same time from some want of oxygen. The

blood was examined and found to contain less than 0,2% urethane, but the exact proportion was not determined.

These experiments seem to warrant the conclusion that the gills of *Astacus* are practically impervious to urethane, though the substance exerts its usual influence when it is brought into the system through the gut or by injection. So far I have met with no other instance of this peculiarity, though it is very probable that more will be found.

Worms.

Nepheleis is slowly but apparently completely narcotized in 1% urethane. It recovers in water.

Nereis is only incompletely narcotized after 6 hours in 1% urethane. It recovers in water.

Molluscs.

Limnaea. Narcosis in 1% urethane after about an hour. Recovery in water. In 0,48% there is incomplete narcosis after 2 hours and after 18 hours the state is about the same. The reflexes are very feeble, but the animal crawls about, though very slowly.

Littorina and *Mytilus* keep closed in a 1% solution of urethane in sea water. After 20 hours *Mytilus* is open. There are spontaneous movements of the mantle, but it closes very slowly on mechanical stimulation.

The chief purpose of the present notice is the practical: To direct the attention of hydrobiologists to a drug which may conceivably prove useful for experimental work on aquatic animals, and I have been prompted to publish it by the fact that a good narcosis of invertebrate animals is often very difficult to obtain by the substances employed hitherto¹⁾.

The action of urethane upon lower animals presents however some points which are also of a certain theoretical interest. It is well known that in mammals the regulation of respiration and circulation is to a great extent detached functionally from the central nervous system, and that narcotic drugs, in concentrations which are sufficient to extinguish all reflexes, will act very slightly or not at all upon the respiration and the circulation.

¹⁾ Bethe, *Wirbellose Tiere* in Tigerstedt: *Handbuch der physiologischen Methodik* 1911, Bd. I, 2, S. 75.

In frogs, fishes and crustaceans (Asellus, Idotea, Daphnia) a concentration of ethyl urethane which will produce complete narcosis will have no visible effect on the heart, just as in mammals, and there is a certain detachment of the respiration also, though that is much less pronounced. It is possible to find for each species a certain concentration, which will extinguish the reflexes completely or almost completely without stopping the respiration, but a slightly higher dose will generally be sufficient to bring the respiratory movements to a complete standstill.

A closer study of these relations, extended to a larger number of forms will probably produce results of considerable interest. I hope to be able to undertake such a study in a not too distant future.