

to higher levels. Implied in these deductions is the principle that sodium is the critical factor limiting vole populations in many areas. In regions with high sodium soils, enough salt may be ingested with the vegetation to satisfy the increased requirements of crowded animals, thereby permitting unrestricted reproduction and population growth to the point where food or some other factor becomes critical.

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Simple Technique for embedding and supporting Delicate Biological Specimens

MANY biological specimens are too large for treatment by normal microscopical techniques and, at the same time, too small and delicate to withstand very much manipulation. Many embedding media have been tried over the past few years, and one of the most efficient and effective is agar.

A 4 per cent aqueous (distilled water) solution of agar ('Bacto', Difco Laboratories) is prepared, either by free steaming in an autoclave or steamer, or by using a beaker suspended in a boiling water bath. The molten agar is cooled to about 40° C and a thin layer poured into a solid watch-glass and allowed to set. The specimen is arranged on the surface of the agar and is then covered by a further layer of molten agar. It is important to ensure that no moisture is left on the first layer. Moisture globules will spread between the two layers and can cause separation after setting. This trouble is obviated by wiping the specimen and the surface of the agar with a small piece of filter paper after the specimen has been orientated. When the agar has set, the lens-shaped block is eased out of the watch-glass and the part containing the specimen trimmed into a cubical block, using a sharp scalpel or razor blade. The cube (which is opaque when set) is then transferred to 70 per cent alcohol, in which it may be stored until required for clearing. For clearing, the block is gradually dehydrated by being taken through 80 per cent, 90 per cent and absolute alcohol, one day in each, and then transferred to a mixture of equal parts of absolute alcohol and benzyl alcohol for a few hours. It is then removed to pure benzyl alcohol for complete clearing. Both block and specimen will become transparent quite quickly but specimens containing a great deal of yolk may require a longer time to achieve complete transparency. In the case of amphibian eggs this is an advantage because the slight opacity gives a clearer picture of the segmentation stages.

The technique is particularly useful for eggs of cyclostomes, teleost fishes and amphibia, coelenterate medusae, dissections of small molluscs and molluscan radulae too large for ordinary microscope mounts. Specimens may be stained before embedding, but they must be returned to water before processing. Blocks may be removed from the benzyl alcohol for examination under a dissecting microscope, but should not be kept in the air for a period of more than 3 h, otherwise splitting and drying of the block may occur. All material for treatment under these conditions must first be fixed and preserved as for normal storage. The blocks are tough and resistant to wear, but should it be necessary to remove a specimen from a block a careful cut across a block in one plane will enable the specimen to be removed without damage. All parings from the first trimming may be remelted and

used for more blocks. Agar cannot be recovered after treatment with alcohol.

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Selective Effect of Particulate Insecticides on *Simulium* among Stream Fauna

Simulium, the vector of onchocerciasis and the cause of considerable nuisance in northern latitudes, is usually controlled by killing its aquatic stages by putting insecticides in a soluble form into the streams where they occur. This kills most of the other insects; the natural predatory suppressors of the *Simulium* larvae—the larvae of some of the stone flies and caddis flies—are removed, and unrestrained recolonization by *Simulium* may occur.

The community of creatures living in fresh-water streams is highly organized and stable and constitutes many food chains, one ending in fish. The use of soluble DDT necessitates repetition and produces an entirely different organization, the maintenance of which is dependent on the maintenance of the interference. This changed environment can be regarded in many respects as biologically sterile.

Simulium larvae are particle feeders. They live only in the fast-flowing parts of the stream, and their method of feeding is different from that of the other detritus feeders such as the chironomids, which live in the slow-running and silted part of the stream. The size of particles ingested by British species of *Simulium* is about 10–12 μ (ref. 1). Since 1962, we have used DDT in the form of particles of different size in streams in North Wales. In one such experiment, 1×10^{12} particles of 4–15 μ were put into a stream in half an hour, when the stream was flowing at a rate of 0.15 cu. ft./sec. This corresponded to a dosage of 0.5 p.p.m. The next day, all *Simulium* larvae had disappeared from polythene tapes placed 150 yards below the point of dosage, and the tapes remained free of *Simulium* until a month after the dosing. During this time no other creatures in either the water or the stream bed showed any population variation other than the minor fluctuations expected from the changing seasons and rainfall. Not even net-spinning caddis fly larvae were affected by the small particles of DDT.

It is possible that a method of control developed on this principle will not disturb the predator insects which regulate the numbers of *Simulium* larvae, and it should not interfere with the tightly knit community of creatures which includes the food chain leading to fish. But we do not know whether the particulate insecticide accumulates in the sediment of the river bed, nor do we know anything of the potential effects of such accumulation, and we know nothing of the fate of that fraction which is taken up by *Simulium* larvae. These and other factors must be investigated.

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