

100–120 mgm. per flask. The extracellular nitrogen formed represented 30–35 per cent of the nitrogen supplied. There was no difference in effect between the two levels of chloramphenicol.

This very marked effect of chloramphenicol on the metabolism of *S. brevicaulis* was only found with nitrate as nitrogen source. When nitrate was replaced by ammonia, nitrite, or a mixture of amino-acids, chloramphenicol was without effect and the fungus behaved exactly as in the control flasks. The effect of chloramphenicol in nitrate medium was also completely removed merely by growing the fungus in submerged conditions with vigorous shaking.

The inhibiting effect of chloramphenicol on the fungus in nitrate medium in static culture could not be overcome by the addition of increased trace elements (up to ten times the normal level), or by the addition of yeast extract. It could, however, be overcome by an unknown factor present in commercial glucose and removable therefrom by a cation exchange resin. There was no evidence that the nitrate reductase system was inhibited in the presence of chloramphenicol.

When several other fungi (*Penicillium griseofulvum* Dierckx, *Botrytis allii* Munn., *Aspergillus niger* v. Tieghem, *Fusarium graminearum* Schwabe) were tested in the same conditions as *S. brevicaulis* their rate of growth and nitrate assimilation, and the extent to which they formed extracellular nitrogen compounds, were completely unaffected by chloramphenicol.

No explanation can be given of this effect of chloramphenicol upon *S. brevicaulis* alone among many fungi tested and manifest only in nitrate medium in conditions of surface culture. It seems worth recording if only as an example of that biological specificity which is often so puzzling.

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### Removal of Osmic Acid Stains

In the current literature on electron microscopical methods<sup>1</sup> many weaknesses of osmic acid as a fixing agent have been carefully analysed and appropriate safety methods, which would reduce if not entirely eliminate these, have been recommended. No useful advice appears to have been given, however, on the proper cleaning of glass and other utensils after they have become stained with osmic acid or on the removal of osmic acid precipitates, and consequently some wastage of this not inexpensive material at present seems to be accepted as inevitable.

Many years ago, one of us<sup>2</sup>, working on an extensive series of peripheral nervous tissue material treated with osmic acid, found bleaching of the sections in 3 per cent hydrogen peroxide extremely useful as a preliminary to the application of other staining methods. Though no special mention was made of this, as it appeared a logical step to take, at that time all glassware and other apparatus contaminated with precipitated osmic acid was washed as a matter of routine with hydrogen peroxide, the strength of the solution depending on the heaviness of the soiling,

or the speed required for the finishing of the cleaning process.

With the advent of electron microscopy, osmic acid is again used extensively, and the need for efficient cleaning of glassware and apparatus has reappeared. Hydrogen peroxide was again found fully satisfactory for this purpose and it is used by us as a routine procedure.

It is well known that osmic acid renders cells and tissues difficult to stain by customary methods. Presumably because of this difficulty most workers, in order to achieve the necessary tissue orientation in electron microscopy, use the phase-contrast microscope for this purpose on parallel, thicker sections, though the information thus obtained is not as good as that provided by stained preparations.

At present we are studying systematically the staining properties of sections fixed with osmic acid and embedded in methacrylate after bleaching them in hydrogen peroxide. It is already apparent that most staining methods are applicable to these sections with reasonable success and without any major modification in the procedure.

Details of this work will be published elsewhere.

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### An Apparently Simple Case of Population Control

It is not usually possible to discover what factors control any particular population, that is, what factors act more strongly against the population as it increases and so tend to keep its numbers constant. In a small planted beech wood just west of Marlborough (Nat. Grid Ref. SU 175680) there is a population of the common snail *Helix aspersa* which seems to be controlled in a very simple manner. The trees have small buttresses at their bases, leaving narrow caves between, and in the winter the snails crawl into the caves to hibernate. When I visited the wood on January 21, 1954, with Drs. S. M. McGee Russell and P. M. Sheppard, we found no *aspersa* were hibernating near the outsides of the caves: all were deeply inside. When Dr. Sheppard visited the copse in the previous autumn there were many snails not fully in the crevices, and he tells me that there were far fewer predated shells than we saw in January. It was as if all snails which were hibernating in a position in which a predator could reach them had been eaten, while those in inaccessible positions survived. As these inaccessible positions would not change greatly from year to year, this system would keep the population at a constant size in the same way that an overflow pipe keeps water in a tank at constant depth. Squirrels were probably the predator concerned.

This colony is unusual not only in its system of control. The snails were more markedly banded than garden snails, and most were immature. By using sticks we managed to secure 134 live snails of which only 30 were adult.

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