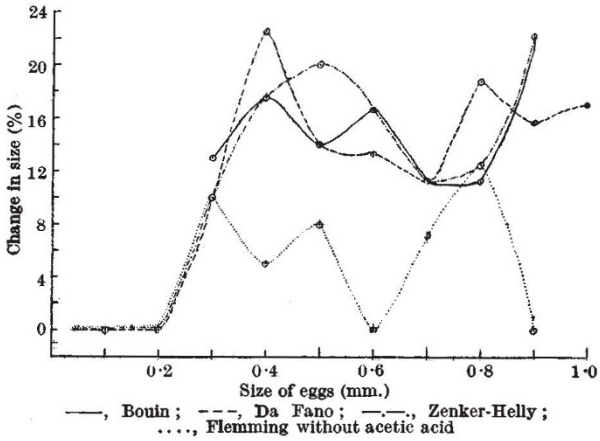

The frequency distribution diagrams on log sectional paper show that the samples taken were not abnormal. Diagrams showing the average shrinkage indicate that shrinkage increases with the size of the egg. The smallest eggs showed almost negligible shrinkage, while in the larger ones it was considerable. The eggs of moderate size also showed a fair amount of change in their dimensions. An interesting point was that in Flemming without acetic acid the eggs swelled up and their diameters increased, although the swelling was less than the shrinkage caused by the other fixatives. Curves of percentage change in dimensions show that of all the four fixatives Flemming without acetic acid seems to be the least harmful; for the highest point on its curve is near the lowest point of other curves.

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The Iron Alum Acetocarmine Method for Algæ

THE chromosomes of many Algæ have been found difficult to stain by the ordinary acetocarmine method. Where staining takes place, it is seldom deep enough in colour. The addition of ferric acetate or other ferric salts to the carmine solution, or rubbing together of steel needles in it, brings about only slight improvement, if any, as observed by Baker[1].

However, the use of iron alum as a mordant on previously fixed material, preceding the acetocarmine treatment, brings about a totally different effect. It has been shown[2] that chromosomes strongly absorb iron from an iron alum solution. They absorb more than other parts of the cell. This, it is thought, must be of importance. With many Algæ, a few minutes or less in a weak solution of iron alum, followed by rapid washing in water, is sufficient for the formation, on addition of acetocarmine, of a dense blue-black colour, presumably ferric carmine lake. This is rapidly dissolved on heating the preparation when covered with a coverglass, but remains longest in the chromosomes, nucleoli and 'chromocentres'. Thus differentiation is brought about. The lake gives a beautifully precise dark red to purple-black stain, and if the degree of mordanting is well adjusted other parts of the cell are only faint pink or scarcely stained. Such preparations are readily made into permanent mounts in 'Euparal' or Canada balsam in the usual way. If the coverglass is floated off in 95 per cent alcohol there is no loss of stain. Preparations which have been kept for two years show no fading.

That the ferric carmine lake can be formed in this way is contrary to the statement of Baker (ref. 1, p. 155), "the lake cannot actually be formed in strongly acid solution". The strength of the acetic acid solution in which the lake appears by the method described above is something less than 45 per cent, due to dilution of the carmine by the small amount of water placed on the slide with the washed algal material: nevertheless this dilution cannot be great. Unless the material has received only a rapid dip in dilute alum solution, there is always an excess of lake to be dissolved away, and it may be necessary to add acetic acid to complete the differentiation.

The contrast with ferric hæmatoxylin lake is marked when aceto-hæmatoxylin is used instead of carmine, after mordanting. On heating, a beautifully differentiated hæmatoxylin stain results, but this is quite transitory. When the permanent balsam mount is made, even after repeated blueing, the colour generally fades within 12 hours.

Other metallic salts, including copper, lead and aluminium, have been tried as mordants. Even other ferric and ferrous salts are, however, apparently less satisfactory than iron alum.

The advantages of the method when used with Algæ are several. It is rapid, a permanent mount being possible within 30 min., starting from fresh material. It can be used following any fixative. Obstacles to the view of the nucleus are readily removed; for example, chlorophyll by fixation with 2 parts of glacial acetic acid to 1 part of 95 per cent alcohol, starch by heating. Not least is the absence of shrinkage, another consequence of heating under a coverglass. Collapse of the cells in xylol is a familiar nuisance when mounting filamentous forms. It is suitable for class-work, when a permanent mount is often unnecessary, for example, for demonstrating the number of nuclei in algal cells. It is equally good for archegonia and antheridia of fern prothalli and has been successfully used on fungi. A disadvantage from the research worker's point of view is its frequent failure after wax embedding.

A detailed schedule of treatment will be included in a forthcoming paper on the nucleolus and nucleolar organisers in *Spirogyra*.

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[1] Baker, J. R., "Cytological Technique" (Methuen, London, 2nd edit. 1945).
[2] Seki, M., *Fol. Anat. Jap.*, **11**, 15 (1933).

Amphibian Tissue Cultures for Biophysical Research

CULTURES of embryonic chick tissue have been successfully used for many types of cytological research and have proved admirable material for biophysical investigations into the mechanism of mitotic cell division[1]. Unfortunately, however, chick fibroblasts *in vitro* are not very suitable for detailed observations on living chromosomes owing to the small size and relatively large number of chromosomes in the fowl.

Previous investigations[2–4] indicated that amphibian cells might prove to be more suitable material for such studies. After some preliminary experiments, a