

a collenchymatous mesoderm, but since it is always necessary to prepare thin slices for the investigation of the finer histological details, but little is gained by this.

Dissociation and teasing must be resorted to for the study of separate histological elements, and the spicules must be isolated from the soft parts for comparison, measurement, and illustration. Under the head of dissociation and teasing I have nothing to add to what is already well known, but with regard to the separation of spicules and the preparation of thin slices, it may prove useful if I describe here in detail those methods which I have found most successful.

*Preparation of Thin Slices.*—The simplest and most generally useful method is that known as the paraffin process; but sometimes in special cases and for special purposes freezing may be resorted to. Although the hard parts of the Tetractinellida are always siliceous, spongin being the only other substance present in the skeleton in addition to opal, yet it sometimes happens that the sponge contains a considerable quantity of calcium carbonate which has been introduced as foreign matter from without, either during the life of the Sponge or subsequently on being scraped up by the dredge; this calcareous matter is usually in the form of fine mud or consists of isolated tests of Foraminifera and other organisms; frequently also calcareous organisms, more especially Foraminifera, grow attached to the outer surface of the Sponge. As a preliminary to staining all traces of calcium carbonate must be removed. This is best accomplished by an alcoholic solution of nitric acid; a 1 per cent. solution of the acid is made with 60 per cent. alcohol, and this is added to the object placed in 60 per cent. alcohol, drop by drop, till an occasional bubble of gas is set free; the preparation is then left to stand for some hours and a further quantity of acid alcohol added till no more carbon dioxide is liberated, the preparation is then transferred to pure 70 per cent. alcohol, which is changed till every trace of calcium nitrate is extracted. The advantage of this method in avoiding any unnecessary osmosis is sufficiently obvious. Fortunately but few of the Challenger specimens needed to be treated in this manner, but "en revanche" in some few cases other mineral bodies, such as fragments of pumice and grains of quartz sand, were present and there is no process by which these can be removed. My experience of hydrofluoric acid, as might *a priori* have been expected, is altogether unsatisfactory. When siliceous fragments occur lying loosely in the canals of the Sponge the razor will generally tear them out, and fairly satisfactory slices may be obtained, as in the case of *Tetilla sandalina*; but when, as in *Psammastra murrayi*, numerous quartz grains occur firmly embedded by fibrous tissue in a dense cortex, one has to abandon all hopes of a thin slice, and to put up with a very bad example of a thick one.

For staining I have found hæmatoxylin most generally useful; picrocarmine and other carmine dyes have also been used, and with picrocarmine especially very elegant results may be obtained; this stain is particularly well adapted for use with the freezing process.