TWO NEW INSTRUMENTS FOR BIOLOGISTS.

By N. A. Cobb.

(Plate vii.)

The Differentiator.*

The differentiator is an instrument which was invented to assist in avoiding to the greatest possible extent those annoying and often destructive contractions which occur in delicate organisms while they are being killed and preserved. These contractions fall into two groups : (1) those which occur before death, due to the action on the organism of the fixation fluids, (2) those occurring during the process of bringing the organism into the fluid in which it is to be finally preserved. Among the latter is an often unavoidable contraction due to transferring to alcohol of successively increasing strength. This contraction takes place with something like equality in the different parts of the organism and is very slight,—much slighter than is commonly supposed, because other contractions or distortions are confounded with it, the whole being denominated shrinkage. Only when the distortions and breakages due to diffusion currents are annihilated does one begin to see how insignificant the unavoidable and true shrinkage really is.

The differentiator is made from glass tubing having an internal diameter varying from five millimeters upwards.

According to the nature of the fluids in use, the instrument takes one of the two forms illustrated in Figs. 1 and 2. As will be seen, *a* or *a'*, the *reservoir*, and *b*, the object-cylinder or *object-box* as well as *c*, the *filter*, are three pieces of glass tubing joined together

* This instrument has been already described in the report (1889) of Prof. Sladen, Secretary of the British Association Committee appointed to manage the Association's table at the Naples Zoological Station. A description also appeared in the American Naturalist, August, 1889. I have since made improvements which render a new description with figures not inexcusable.

by means of caoutchouc tubing. The filter, *c*, is easily made as follows: take a piece of glass tubing twice the length of the required filter, heat it red hot, draw it out to arm's length and break it in two in the middle; remove all the capillary part except about three inches on each half, heat, and bend into the required form *(c)*; next carefully heat the capillary portion near its extremity in a small alcohol flame, draw out exceedingly fine and break off so as to leave a minute orifice. All sharp edges should be rounded off by heating.

To use the differentiator, proceed as follows. Suppose objects fixed by corrosive sublimate are to be studied in balsam after staining with borax-carmine. Fill the filter with perfectly clean sublimate solution and insert a plug of cotton (previously boiled in water to remove the air) at the U-bend (*v*, Fig. 3). Join the object-box to the filter, fill up with sublimate solution, and push a plug of cotton into the lower end of the box, avoiding bubbles. Wrap the cotton in fine linen if the objects are minute. Put the objects into the box, plug the upper end in the same manner as the lower end, and finally join the box and filter thus filled to the empty reservoir *a*, and hang the instrument up in the position illustrated. The objects are now transferred to 33 per cent. alcohol in the following manner: mix equal parts of sublimate solution and 33 per cent. alcohol (call this mixture 2). Mix equal parts of 2 and sublimate solution (call this 1). Mix equal parts of 2 and 33 per cent. alcohol (call this 3). Add mixture 1 to the reservoir until it is one-fourth full, mixture 2 until it is half full, mixture 3 until it is three-fourths full, and then fill up with 33 per cent. alcohol. If the successive mixtures are added with sufficient care, they will, owing to difference in specific gravity, remain distinct. If forced rapidly in, a nearly uniform mixture of about equal parts sublimate solution and 33 per cent, alcohol will result. The desirable procedure lies between these two suppositions, and gives rise to a uniform gradation or *differentiation from sublimate solution to 33 per cent. alcohol in passing upwards through the reservoir*. A good procedure which always secures this result is to add the successive fluids carefully and then to dance

a long fine wire up and down in the reservoir for a few seconds. The flow, which at once commences drop-wise from the point of the filter, should be so regulated, either by tipping the instrument, or by breaking off more or less of the capillary part of the filter, as to cause the reservoir to be emptied in from two to five hours, when the objects will of course be in 33 per cent. alcohol, having been *guarded to the utmost* against diffusion currents. They are now to be transferred to borax-carmine, a fluid *heavier* than 33 per cent. alcohol, by the use of reservoir *a'*. Fill the long arm of the reservoir with borax-carmine, and cork it, leaving the short arm empty and open. Mix equal parts of carmine and 33 per cent. alcohol (call this mixture 2). Mix equal parts of 2 and carmine (call this 1). Mix equal parts of 2 and 33 per cent. alcohol (call this 3). Add mixture 1 to the short arm of the reservoir until it is one-fourth full, mixture 2 until it is half full, mixture 3 until it is three-fourths full and fill up with 33 per cent. alcohol. Transfer the object-box and filter to *a'*, avoiding bubbles, and uncork the long arm. The flow begins as before, but this time *upward* through the object-box, and the objects are thus transferred in from two to five hours to borax-carmine.

After staining, the objects are transferred to 50 per cent. alcohol by means of a reservoir *a*, the flow being so regulated that the change takes place in from ten to twenty hours. Then change successively to acidulated 70 per cent., 90 per cent., and absolute alcohol, allowing at least ten hours for each change. Transfer to turpentine, chloroform, oil of cloves, or any oil desired by reservoir *a'*. Finally to thin balsam, *still by means of the differentiator.*

Whenever the objects are to be transferred to a lighter fluid, use reservoir *a* ; whenever they are to be transferred to a heavier fluid, use reservoir *a'*. If objects are to be transferred to glycerine, transfer first to 50 per cent. glycerine in twelve hours, and then to pure glycerine in twenty-four hours.

Objects which defeat successful fixation by untimely contractions may be rendered insensible by means of the differentiator, and then fixed *perfectly outstretched.* Transfer them in the differentiator to alcohol of from 5 to 30 per cent. (or other

paralyzing solution such as chloral hydrate) in from two to three hours, when they will be insensible and outstretched, and may be fixed as desired. A specially large object-box is easily contrived for larger specimens.

If the objects to be treated are very minute, I now arrange the object-box as illustrated in Fig. 4. Taking a piece of glass tubing, whose external diameter is slightly less than the internal diameter of the object-box, I cut therefrom two pieces, each about 5 mm. long, and round off their sharp edges by heating. Call them rammers. I then cut from the finest linen some circular patches having a diameter slightly greater than the external diameter of the object-box. The next operation consists in taking one of the rammers and with it forcing a wetted patch into one end of the object-box. (Fig. 4, *d*, *e*.) To do this successfully, care should be taken to arrange the patch concentrically on the end of the object-box, and to place the rammer precisely upon the middle of the patch. The patch is to be forced in only so far as is represented in Fig. 4. The rammer may be allowed to remain in, or, if the fit has been a snug one, may with safety be removed, the patch remaining in place by virtue of its own elasticity; the latter is the preferable procedure. Join the object-box, open end uppermost, to the filter, fill up with the fixation fluid, put in the objects, and arrange the upper end of the box with a patch and the remaining rammer. Bubbles must be avoided. The pieces of linen give much less trouble in the end than plugs of cotton, in which minute objects are liable to get lost. In this manner I have treated such minute organisms as rotifers, paramaecium, &c., without loss of specimens. The most delicate organisms return from this treatment in perfect condition.

The flow from the capillary orifice of the filter is best regulated by varying the inclination of the instrument. The flow is fastest when the instrument hangs in a vertical position, and decreases as the position of the instrument is made to approach the horizontal. When the instrument is in a horizontal position there is no flow. A convenient means of varying the inclination of the differentiator

will be found in the rack now to be described. A strip of wood, whose length must be regulated by the number of differentiators it is desired to use, has driven through it, at intervals of from twelve to eighteen inches, long wire nails, whose points will then project an inch. The strip of wood so prepared is fastened in a horizontal position with the nail-points also in a horizontal position. Take a large cork, perforated to fit, and slide it on to the differentiator reservoir. Move the cork back and forth until the whole instrument will balance when left free with the cork resting on the fore-finger—in short, place the cork opposite the centre of gravity. If the cork in this position be impaled upon one of the nails, the latter will be found to afford a good pivot for all movements necessary in regulating the flow.

I formerly gave the long arm of the filter the same diameter as the short arm. Later I found it better to make the filter in the form shown in Fig. 3, *i.e.*, with the long arm semicapillary. When the diameter of this part of the filter was large, space was given for the mixing of the fluids which passed into it, the order of the liquids obviously having a tendency to become reversed after passing the U-bend of the filter. This gave rise to two difficulties. First, when the filter was reversed on changing to the alternate reservoir, a small backward diffusion took place which was especially inconvenient after dehydrating with absolute alcohol; secondly, the precipitate which occurs when carmine fluids are mixed with alcohols had a tendency to accumulate in a flocculent condition in the long arm of the filter, a fact due no doubt to the diffusion constantly taking place there, and this precipitate sometimes clogged the orifice. Both difficulties are avoided in the new form.

The remedy for clogged orifice may as well be given here. If only the extremity is clogged, break away a little of the capillary part, which should always be long to allow for this emergency, and readjust the flow if necessary. If that procedure be inadmissable, hold the point of the filter for a fraction of a second in a small alcohol flame. The slight explosion which occurs will often remove the obstruction.

As regards the mixtures used in differentiating, I formerly made them as they were required. I now keep a stock of fluids on hand. My present stock I find to be as follows:—

I.—*The objects are to be mounted uncut in balsam.*

Alcohols of the following strengths:—7%, 15%, 22%, 30%.

Mixture three parts 30% alcohol and one part borax-carmine.

Mixture one part 30% alcohol and one part borax-carmine.

Mixture one part 30% alcohol and three parts borax-carmine.

Borax carmine.

Mixture three parts borax-carmine and one part 60% alcohol.

Mixture one part borax-carmine and one part 60% alcohol.

Mixture one part borax-carmine and three parts 60% alcohol.

Alcohols of the following strengths:—60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, acidulated as may be necessary.

Mixture of three parts absolute alcohol and one part clove-oil.

Mixture of one part absolute alcohol and one part clove-oil.

Mixture of one part absolute alcohol and three parts clove-oil.

Clove-oil.

Mixture three parts clove-oil and one part thin balsam.

Mixture one part clove-oil and one part thin balsam.

Mixture one part clove-oil and three parts thin balsam.

Thin balsam.

In all twenty-eight.

II.—*The objects are to be cut.*

Go to absolute alcohol as in I.

Then, mixture three parts absolute alcohol and one part chloroform.

Then, mixture one part absolute alcohol and one part chloroform.

Then, mixture one part absolute alcohol and three parts chloroform.

Chloroform.

Then use Giesbrecht's method or some equivalent one for imbedding in paraffin or all previous precautions against shrinkage will have been useless.

III.—*Alcoholic carmine is to be used.*

Go to 60% alcohol as in I.

Then mixture three parts 60% alcohol and one part Mayer's carmine.*

Then mixture one part 60% alcohol and one part Mayer's carmine.

Then mixture one part 60% alcohol and three parts Mayer's carmine.

Mayer's carmine.

Mixture three parts Mayer's carmine and one part acidulated absolute alcohol.

Mixture one part Mayer's carmine and one part acidulated absolute alcohol.

Mixture one part Mayer's carmine and three parts acidulated absolute alcohol.

Absolute alcohol.

Then the appropriate clove-oil and balsam mixtures.

IV.—*The objects are to be preserved in glycerine.*

Glycerine of the following strengths, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%.

When turpentine and such other fluids as act on caoutchouc are used, it becomes necessary to use stout caoutchouc tubing and to tie it on firmly.

The Suction-capsule.

The suction-capsule was devised to aid in solving those difficult and important problems connected with the development in the human alimentary canal of the eggs and larvæ of the internal parasites peculiar to man, and incidentally to serve in a similar manner in investigating the internal parasites of the lower animals.

The usual procedure (feeding the eggs to a subject direct and recovering them or the resulting larvæ by killing the subject) is not applicable to man, and is in any case open to some serious objections, especially in certain cases. It is often impossible to

* I make this carmine to contain 80% alcohol and ensure the exact percentage of alcohol by making in a flask immediately connected with a vertical Liebig's condenser. No alcohol is then lost by evaporation during the boiling.

be certain that the eggs or larvæ recovered are identical with those administered, and this alone is a serious drawback. With man himself the case stands most precarious. To show this it will suffice to cite one of the best known and most decisive experiments of the kind under consideration. Prof. Rudolph Leuckart, the celebrated Leipzig naturalist, swallowed a number of the eggs of *Oxyuris vermicularis*, the common pinworm or threadworm of man. About two weeks later he passed some nearly mature worms of that species, as it seems did also several of his pupils, who performed the same experiment simultaneously with him. The perfectly obvious conclusion is that the worms noticed were those resulting from the eggs wittingly swallowed two weeks previously, providing the experimenters were previously free from *Oxyuris*. But the proviso seriously mars the result. The more familiar one is with the abundance of the eggs of *Oxyuris vermicularis*, and the multitudinous chances they have unbeknown to him of getting into the alimentary canal of man, the more one will hesitate in admitting that more than a probability was established by Leuckart's experiments. No one could be better aware of this than the renowned author of the "Parasites of Man," and his statements are therefore only those justified by his experiments. Succeeding authors have not been correspondingly careful, and one may find the statement in what seem to be authoritative places boldly made on this very evidence, that the direct development of *Oxyuris vermicularis* has now become certain. Balfour very properly qualifies the "certain" by prefixing "almost."

The suction-capsule will, I hope, lead to a greater amount of certainty in this field.

The idea embodied in the suction-capsule occurred to me two years ago at Jena. At that time I made some experiments with it, but they came to no satisfactory conclusion as the sequel will show. Recently while making some researches into the life-history of certain Australian entozoa I tried it again and this time with most gratifying results.

The suction-capsule is made from thin glass tubing of two to five millimeters external diameter. A capsule adequate for experiments on lower animals is easily made. Connect an ordinary blow-pipe with a filled air-bag and produce a narrow oxidizing-flame by sending a blast from the blow-pipe through the flame of a small alcohol lamp. Heat the glass tubing successively in two places and draw it out into the form shown in Fig. 5. The length of the central capsule, *s*, should not much exceed twice its width. Now break off the tubing at one end of the capsule and heat that end in the blow-pipe flame until the aperture becomes minute, and follow this operation by heating and drawing out the narrow tube at the other end of the capsule into the form shown at *t*, Fig. 6. The glass at *t* will remain tubular. Suppose the minute aperture at *u*, Fig. 6, to be stopped with glue. Then if suction be exerted at *r* and while the suction is being exerted the tube at *t* be melted in a fine-pointed blowpipe flame, the capsular portion will be severed from the remainder of the tube shown in Fig. 6, and will constitute what I call a suction-capsule. (See Fig. 7.) Such a capsule contains a partial vacuum, and has the power under appropriate conditions of exerting suction. This characteristic property is soon observed if the capsule be immersed in water. The plug of glue soon dissolves and water is then drawn into the capsule in quantity proportional to the previous exhaustion of the air. For instance

a capsule which weighed	108 mg.
on being filled with water weighed	230 mg.
Hence the capsule was capable of holding ...	·122 cc.
After partial exhaustion of the air and immersion in water the weight was	150 mg.
Hence the water sucked in weighed	42 mg.

In other words this suction-capsule when immersed in water sucked itself about one-third full. This completely illustrates the mechanical action of the capsule and it now only remains to say a word about the manner of its use. It is made the vehicle for conveying to the stomach of animals, man included, the eggs of entozoa. On reaching the stomach the capsule takes in gastric

fluids and the eggs are therefore subjected to the action of those fluids under normal conditions. The capsule is recovered from the fæces in the case of man but in any way desired in the case of lower animals. The results leave little room for the distressing uncertainties often attendant upon simply feeding the eggs. On recovery the contents of the capsule are invariably acid. The inserted eggs are therefore subjected to the action of the gastric fluids only.

The eggs experimented upon are put into the capsule either through the aperture shown at *u*, Fig. 6, before it is stopped with glue, or are introduced through *t* after *u* has been stopped. In this latter case *u* may be made so small that no resulting embryo can escape, a result which may also be secured by tying filter paper over the aperture *u* by means of cloth and a strong fine thread. A pipette may be made with an exceedingly fine capillary neck longer than the distance from *u* to *r*, Fig. 6, and by means of this pipette eggs (in water) inserted into the capsule through *t* after *u* has been stopped. Fusing the capillary part *t* does not heat the capsule sufficiently to injure living eggs.

There is a choice of ways in exhausting the air. One may attach a rubber tube at *r* and by sucking ones utmost thereon produce a sufficient exhaustion. I use a pair of two-quart bottles containing water and connected by a long piece of rubber tubing arranged so that by lowering or raising one of the bottles by means of a cord and pulley a variable pressure or suction can be exerted at will. I have not found it expedient to reduce the pressure in the suction-capsule to less than three-quarters of an atmosphere, an effect which one can barely produce with no other pump than one's own lungs.

Capsules varying from 1 millimetre to 4 millimetres may be easily fed to many animals and a little ingenuity will succeed in introducing them almost anywhere desired. I have repeatedly swallowed such capsules and have never experienced any inconvenience beyond a nervous anxiety at the first trial.

As a small proof of the usefulness of both the instruments here described, I accompany this paper by another entitled, "Oxyuris-larvæ hatched in the human stomach under normal conditions."

EXPLANATION OF THE FIGURES.

a.—Reservoir used in transferring to lighter fluids.

a'.—Reservoir used in transferring to heavier fluids.

b.—Object-box, or object-cylinder.

c.—Filter.

d.—Short piece of glass tubing.

e.—Patch of finest linen.

s.—Capsule.

t.—Capillary neck to capsule, fine to be fused.

u.—Mouth of capsule stopped with glue.

v.—Plug of cotton.

Figs. 1 and 2.—Differentiator, one-fifth convenient size, with the three parts *(a, b,* and *c)* joined in place by caoutchouc tubing.

Fig. 3.—Object-box *(b)*, and filter *(c)*, full size, showing the best form of filter.

Fig. 4.—Object-box, full size, showing best method for securing minute objects.

Figs. 5 and 6.—Two stages in the process of making a suction-capsule.

Fig. 7.—Suction capsule ready for use, magnified ; seen in section.