locum habeat. Ab hoc ulteriore differt forma et directione et colore stromatum et peritheciis non prominulis.

99. PYRENULA ADACTA, *Fée*, *Ess.* p. 74.

100. ANTHRACOTHECIUM MANIPURENSE, *Müll. Arg.*; thallus flavescenti-pallidus, tenuissimus, lævis; apothecia atra, $1\frac{2}{3}$–$1\frac{4}{5}$ mm. lata, sessilia, nuda, hemisphærica, nonnihil conico-acutata, vertice minute umbilicato-ostiolata, opaca; perithecium basi valide completum, subtus convexum, lateraliter basi valide productum; sporæ 3(–4)næ, fuscæ, circ. 110 μ longæ et 37 μ latæ, crebre cubico-parenchymatosæ, series transversales 5–6-locellares.—Juxta Brasiliense *A. aurantium*, Müll. Arg. Revis. Lich. Eschw. n. 7, locandum est, a quo non diversum videtur nisi thallo minus aurantiaco-fulvo, apotheciis paullo altius convexis et sporis majoribus. Apothecia distincte minora et convexiora sunt quam in *A. Thwaitesii*, *A. borbonico* et *A. macrosporo*, Müll. Arg.

101. A. VARIOLOSUM, *Müll. Arg. Lich. Afric. occid.* n. 52.—Pyrenula variolosa, *Pers. in Gaudich. Uran.* p. 181.

STUDIES IN VEGETABLE BIOLOGY.—VIII. An Investigation into the True Nature of Callus.—Part II. By SPENCER LE M. MOORE, F.L.S.

[Read 18th June, 1891.]

(PLATE XXV.)

Examination of the Fig.

IN the soft bast of the Fig two methods of closing the sieves of the sieve-tubes are to be noted: the sieves lying upon the abaxial side of the bast are obliterated by means of a substance giving the ordinary dye-reactions of callus, and notably the distinctive Cambridge blue with picric Sands' sblue (Pl. XXV. fig. 1, *a*). Many of the sieves of tubes lying upon the axial side of the bast are clogged with a substance of glassy appearance and dense consistence, often possessing a yellow refringence; this does not stain with picric blue, but, like the stoppers of *Ballia*, which, when occurring in the form shown at fig. 1 *b*, it at once recalls to mind, takes a yellow colour with that reagent. It will not be necessary to give in detail, as was my first intention, all the experiments upon the callus of the first-mentioned tubes, made primarily with the object of finding out whether it will give proteid reactions and will peptonize. The results obtained may thus be epitomized:—

1. The callus rapidly dissolves on warming sections in Millon's fluid; and does so without showing any tendency to become red.

2. It is soluble in boiling hydric nitrate; hence the xanthoproteic test does not succeed.

3. On running in caustic potash after sections have lain some time in copper sulphate, the callus swells up, but does not turn blue or pink.

4. After a good soaking in syrup, hydric sulphate swells the callus so that it is almost invisible, but it never assumes the slightest tint of pink.

5. After many experiments with a peptic fluid, allowed to act as long as 86 hours, the callus underwent not the least change in form and general appearance; and it now reacted quite normally with picric blue and corallin soda.

6. The same result followed every attempt to dissolve the callus in a pancreatic fluid (Fairchild's pancreatic extract).

On the other hand, the substance closing the sieves of the inner tubes behaves thus:—

1. It stains yellow with picric blue.

2. It takes a temporary pink with corallin soda.

3. In hydric sulphate there is no appreciable swelling up, nor in caustic potash.

4. Iodine stains it brown.

5. It is unacted on by carmine.

6. It gives good proteid reactions.

7. On many occasions it dissolved in a peptic as well as in a pancreatic fluid.

This substance therefore greatly resembles the substance of Ballia stoppers; at the same time it is quite different from callus in its reactions and chemical constitution, although the same function of obliterating the sieves is performed by both. I propose to call it "paracallus," the term callus being an unsuitable class-name to include both a proteid and a non-proteid substance.

Re-examination of the Vegetable-Marrow.

Having collected the foregoing results from the Fig, and remembering that the substance staining like callus discovered by F. W. Oliver in the trumpet-hyphæ of *Macrocystis pyrifera*, Ag., refuses to give proteid reactions and to peptonize*, re-examination of the Vegetable-Marrow seemed desirable. But very little progress had been made, when I found that I had been unfortunate in basing my conclusions with regard to Vegetable-Marrow callus upon observations which, although perfectly correct in themselves, were yet in a measure valueless, from having been made with abnormal material. I have preserved two sections derived from that material, each of them showing a considerable number of sieves all cleared after the action of a peptonizing fluid, and without the slightest suspicion of callus anywhere; and this is an obviously abnormal occurrence, in view of the undoubted fact that the true callus of the Vegetable-Marrow will not peptonize and will not give proteid reactions. In fact, it behaves in the latter respect just as does the callus of the Fig and of *Macrocystis*, either swelling up almost to invisibility, or dissolving with great promptitude. The statements made in the former memoir must therefore be understood as applying not to the true callus, but to the proteid callus, *i. e.* the paracallus; and I believe the latter substance to occur much more frequently in the Vegetable-Marrow than the former, at least that is the inference suggested from the material at my command.

Concerning this paracallus, a few more facts have been made out. In the former memoir its affinity with coagulated proteid was suggested, and this seems borne out by its behaviour with nickel sulphate and ammonia. This is Gnezda's † test for proteids; albuminates, globulins, fibrin, and mucin giving a pale-blue solution with it, while coagulated proteid is turned yellow.

Now if sections of Vegetable-Marrow bast be allowed to lie in Gnezda's fluid, the paracallus-masses will be found to be bright yellow, a colour which becomes somewhat more pronounced on running in caustic potash; and this, according to Gnezda, is a further peculiarity of coagulated proteids ‡. Another matter

* As was shown in my former memoir on callus (Journ. Linn. Soc., Bot. vol. xxvii. p. 519). † Proc. Roy. Soc. vol. xlvii. (1890).

‡ In one case a distinct blue colour was taken by a paracallus-mass; perhaps this indicates a mixture of proteids in paracallus.

worth mention is the great difficulty met with in peptonizing the paracallus: this I suppose due to the fact that the spirit in which the material was kept was found, upon examination, to have evaporated, so that upon the dehydration undergone from the action of the spirit desiccation had supervened; and this might well tend to make the paracallus less soluble. Anyhow, be the reason what it may, I now found it impossible to entirely dissolve the paracallus in a peptic fluid; and although a pancreatic fluid was more efficient, it failed in a few cases. This should teach caution in the attempt to classify these proteids upon results yielded by preserved material: for this reason I wish it to be understood that the comparison between the substance of *Ballia* stoppers and lardacein, drawn in the former memoir, is to be regarded as merely provisional.

That an undoubted proteid should, apparently because of the dense aggregation of its particles, refuse to answer to those distinctive proteid tests—the taking up of aniline-blue and of carmine—is a singular fact; it is very often the case with the typical paracallus studied by me. Another seeming consequence of this density is the peculiar appearance borne by some sieves ("hedgehog" sieves they might be called) of desiccated Vegetable-Marrow material: the meshes of these sieves are blocked up with the hard paracallus which in surface-view projects therefrom in the form of a number of hard bristly points (Pl. XXV. fig. 7); these, like the paracallus mass itself, disappear in an effective peptonizing fluid.

The Ash.

This is a good type to study, on account of the great development of its callus: the obliterated sieves are shown, Pl. XXV. figs. 2 and 4*a*. Occasionally one may find a few sieves open during the winter; but for the most part they are blocked up with oval masses of callus presenting the normal reactions. I have not found paracallus on any of these sieves.

After many experiments, no encouragement has been given to the idea that this callus may yield proteid reactions; all the masses behave exactly as do those of the abaxial tubes of the Fig. Moreover, the callus resists both gastric and pancreatic digestion.

The Dog-Rose.

Most of the sieves of one-year shoots of *Rosa canina* are, during the winter, blocked up with masses of paracallus. Examples of this are shown, Pl. XXV. figs. 3 *a*, *b*, *c*, and *d*. Of these, *a* represents a small mass; *b* one upon a side-wall, seen in profile; *c* a paracallus-cap, recalling a *Ballia*-stopper, hanging by paracallus-(?) threads to the sieve-plate from which it has been disturbed by sectioning; at *d* is drawn a large mass blocking two sieves from which sectioning has partly dislodged it. This paracallus gives good proteid reactions; and in some cases efforts to peptonize it met with success, though failure was sometimes experienced even after lengthy action of a gastric or pancreatic fluid.

Some General Remarks.

Experiments were also made with the Elm, with *Ampelopsis hederacea* and *Veitchi*. Of these, the former resembles the Ash; for I could find no paracallus upon its sieve-plates; while the two latter are more like the Dog-Rose inasmuch as paracallus is frequently seen in them. The callus of the rhizome of *Arundo Phragmites* is also true callus; though unfortunately, from its paucity upon the sieves of my material, I have not been able to make a thorough examination of it.

It might perhaps be objected that the paracallus is merely that somewhat denser condition of the slime met with at one or both ends of the sieve-tubes—usually at one end—and called by German writers "Schleimkopf." It undoubtedly is formed from the Schleimkopf, towards which it seems to bear the same relation that the Schleimkopf itself does to the slime. This is well seen in large cells of *Ballia*, in which we often find aggregation of the contents at one or at either end of the cell, which is capped by the paracallus-stopper. Apart from its function of obstructing the sieves, paracallus differs from Schleimkopf in its so frequent refusal to take up carmine and aniline-blue. Its chief differences from true callus have already been explained, though in this connexion one matter of interest must now be mentioned. Both callus and paracallus stain well with watery eosin; but whereas the stain taken by the former is not permanent, that imparted to the latter is so. I recently came across a beautiful instance of this. A few years ago I mounted, under one coverslip, some large sections of Vegetable-Marrow bast showing well-developed callus; these had been stained some in watery eosin, others in picric Sands's blue, and happening recently to examine the slide, I found that an interchange of stain had taken place between callus and paracallus, the callus of eosin sections having given up its eosin and taken the blue of the other sections, while the paracallus had monopolized the eosin and had become deeply dyed with it. Of course it is usually fairly easy for the practised eye to distinguish between callus and paracallus; and if there should be any doubt on the point, it can soon be dissipated by the use of picric blue, with which callus stains a Cambridge, and paracallus (if at all) an Oxford-blue. I experienced most difficulty in dealing with the Dog-Rose, masses which in this case I thought to be callus often proving to be paracallus, and *vice versâ.*

We thus see how, *à propos* of the methods whereby the sieves are closed up, we are able to write down the following series:—

In *Ballia* we have paracallus alone.

In *Rosa canina*, *Ampelopsis hederacea* and *Veitchi*, the Fig, and *Macrocystis pyrifera* we have callus and paracallus.

In the Ash and the Elm we have callus alone.

Is True Callus Soluble in any Organic Fluid?

We can frequently get information about the nature of a substance by studying its solubilities, and this consideration led me to try the effect of diastatic ferments upon callus. The method consisted in introducing thin radial longitudinal sections into vessels containing the ferment, these being kept for some time at a temperature higher than that of the air, and generally at, or a little below, that of the human body. I have already mentioned the want of success in working with a pancreatic fluid, and as this, besides trypsin and steapsin, contained diastase as well, we are forced to conclude that callus is untouched by a diastatic ferment. This conclusion was borne out on treatment of Fig, Ash, and Elm sections with saliva for 60 hours, and for 140 hours with Kepler's malt extract, which contains a powerful diastatic ferment.

I then turned to gum-arabic with the idea that, as callus is generally supposed to be of mucilaginous nature, a solvent for it might possibly be found in the gum. In this surmise I was not mistaken; and I have several times successfully repeated my observations, which were made especially upon the Fig and the Ash. Reference to Pl. XXV. fig. 4 will bring to the eye what happens when a section of Ash-bâst is placed in a solution of gum-arabic; in this case the action lasted for 49 hours at or near the temperature of the body. We see how the callus has disappeared, leaving the sieve quite clear, leaving also in position the thin film of Schleimkopf which was closely apposed to the callus-mass. Fig. 5 shows what occurs in the Fig: here there was no film of Schleimkopf, and all one sees is the perfectly cleared sieve. Moreover, as showing the identity between Vegetable-Marrow callus and that of the Fig and Ash, it was found that the former substance also dissolves in gum-arabic. By this treatment one can get beautiful preparations showing cleared sieves with slime-threads passing through the pores, and so connecting the protoplasts: this is shown by fig. 6, where the sieve is seen free from callus, the Schleimkopf (*s*)—a large mass on one side, and greatly reduced on the other—remaining with the same contour that it had when callus was present, and the connecting-threads, around which the callus was deposited, remaining in their original position. These facts may be commended to anyone who favours the swelling-up theory of callus-formation, a theory which they seem emphatically to contradict.

Search for a Callolytic Ferment.

How is callus dissolved away from the sieve-plates? Most probably in one of two ways, either by means of a ferment or of an acid or alkali—possibly by cooperation of ferment with acid or alkali. I regret being unable, after spending some months upon this question, to give it a definite answer; and the case being thus, I shall not go into my various experiments in detail. If there be a callolytic ferment in gum-arabic, it must act in presence of an acid, for a solution of the gum has a decided, though not a very strong, acid reaction. Working with gum-arabic solution, I tried the precipitation method of ferment-isolation, but without any success; and the same failure followed all the experiments with glycerine extracts of crushed gum. The soft bast of the Ash gives a feebly acid reaction—at least in early spring; and this seems to indicate that the contents of the sieve-tubes are acid. With this type also both methods were tried—precipitation and glycerine-extract; but all was labour in vain. But I have not yet tried with gum-arabic the method of dialysis, which may possibly bring success in its train.

Some Remarks on the Function of Callus and Paracallus.

In the former memoir I adopted Sachs's view that the function of callus is to act as a mechanical hindrance to the flow of proteid &c. along the sieve-tubes. I do not know to what length Sachs would carry this idea, neither am I aware of any attempt to think out fully the effects which one would expect to occur were the sieve-plates not blocked up in winter with callus or paracallus, or some other substance with a similar function. Let us suppose the flow along the sieve-tubes to be absolutely without hindrance; how, then, could our arborescent vegetation survive? In these latitudes scarcely a winter passes without some days of premature warmth, during several hours of which the mercury rises far above the minimum point, and sometimes stands not very much below the optimum temperature for growth. Under these circumstances a certain amount of activity manifests itself at the vegetative points: leaf-scales elongate, and may betray a tendency towards epinasty, thus partially exposing the tender young leaves; and should the weather continue warm, the infant axes may begin to lengthen only to be destroyed with the recurrence of seasonable weather. Now if this can happen when the growing-points are shut off from access to the stores of proteid and carbohydrate pabulum accumulated in them, what would be the upshot if these stores were not safely locked up? Were this the case, we may be sure that a fortnight's warm weather in winter would witness an astonishing advance—growing axes would elongate, leaves would stretch and unroll, probably many flowers would expand; and all the energy used up over this would be so much dead loss to the plant, and worse than dead loss, because more energy would have to be drawn upon for the production of new meristematic points to replace those cut off. A healthy tree would doubtless respond to the call upon it, and during its first season, under the altered conditions, would show but faint signs of a change in the order of nature. Its store of accumulated energy would, however, be less during the following than it was the preceding winter; and this diminution would, irrespective of the loss from precocious sprouting, betray itself next year in the production of fewer and probably of smaller leaves. In fact, our supposed tree would be in the position of a person living to some extent upon his capital. We may be certain, therefore, that but for these microscopic pieces of callus and paracallus, a few years would bring the life of our trees to an end, and the wayfarer would be saddened on seeing the monarchs of the forest stripped of all their glory and everywhere hastening to decay.

And as with temperature, so with moisture. But for callus an occasional shower or two during the dry season would stimulate a tropical tree's growing-points into activity only for the young shoots to perish upon renewal of the drought. Nor should the agency of light pass out of view: we know that callus is remarkably developed in climbing-plants, and were the flow of pabulum not carefully controlled, and so made available for the effective organs of the plant, viz. those which have reached the light, we may readily conceive how proteids and carbohydrates would tend to accumulate at imperfectly illuminated points, and how in consequence new and useless shoots would be formed there. In short, the presence of callus and paracallus prevents the expenditure of energy over the production of organs in unfavourable situations, or under conditions which, although favourable, are only temporarily so.

Summary.

1. The statement made in the previous memoir to the effect that Vegetable-Marrow callus gives proteid reactions, and will peptonize, is an error due to the misfortune of working with abnormal material. Some of the sieve-plates in the Vegetable-Marrow are obliterated by true callus which neither gives proteid reactions nor peptonizes; others at the end of the season are blocked by the proteid body studied in the former memoir. This latter substance it is proposed to call "paracallus."

2. On the abaxial side of the bast of the Fig the sieve-plates are closed-in in winter with a substance which refuses to give proteid reactions and to peptonize: upon many of the inner plates there is a hard proteid mass which frequently peptonizes. The first substance is true callus; the second (paracallus) is a further hardening of the Schleimkopf, and, when best developed, is characterized by taking up neither carmine nor picric blue.

3. In *Ballia* we find paracallus alone; *Rosa canina*, *Ampelopsis hederacea* and *Veitchi*, the Fig, and *Macrocystis pyrifera*

have paracallus as well as callus: while callus alone occurs in the Ash and the Elm.

4. True callus resists the action of diastatic ferments, but dissolves in a solution of gum-arabic; hence there is probably a callolytic ferment in the gum, although efforts to find it have so far been unsuccessful.

5. The function of callus and paracallus is to protect the plant by preventing the formation of new shoots under only temporarily favourable conditions of heat, light, or moisture.

EXPLANATION OF PLATE XXV.

All figures magnified 600 times.

Fig. 1. The Fig: *a*, callus of abaxial sieve-tubes; *b*, *c*, paracallus-masses upon the sieve-plates of the inner tubes.

2. The Ash: sieve-plate plugged with a mass of callus.

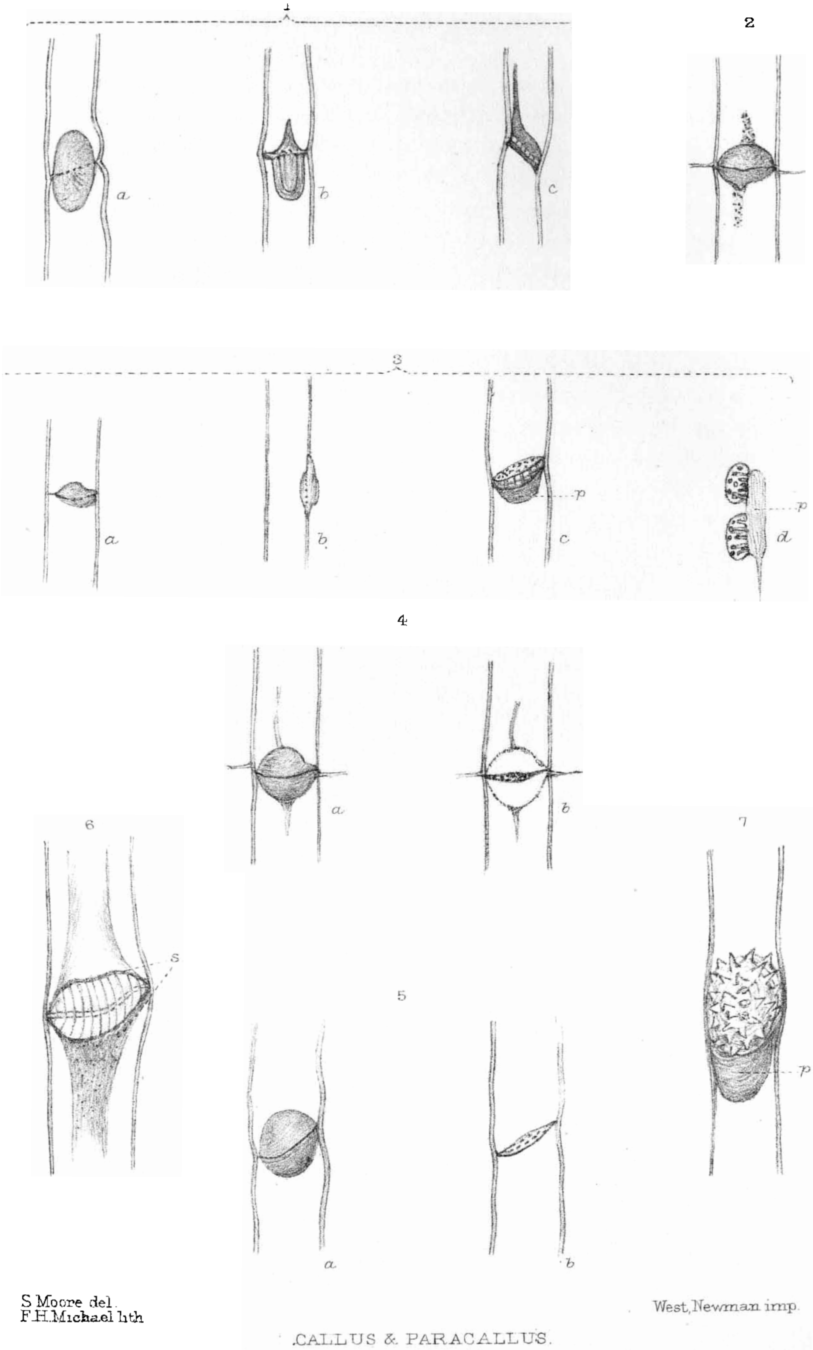
3. *Rosa canina*: *a* and *b*, small paracallus-masses upon sieve-plates; *p* in *c*, the paracallus-cap, in *d* a mass common to two sieve-plates.

4. The Ash: *a*, before, *b*, after dissolution of the callus in a solution of gum-arabic.

5 *a* & *b*. The same thing, shown in the Fig.

6. Effect of a gum-arabic solution on the callus of the Vegetable-Marrow: *s*, the Schleimkopf of contiguous sieve-tubes connected by threads of slime passing through the meshes of the cleared sieve-plate.

7. The Vegetable-Marrow: a "hedgehog" sieve: the hard paracallus projects from the meshwork in a number of points; *p*, the mass of paracallus applied to one side of the sieve-plate.



S Moore del.
F.H. Michael lith

West, Newman imp.

CALLUS & PARACALLUS.