On some points in the Histology and Physiology of the Fruits and Seeds of Rhamnus.

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With Plates I and II.

SOME time ago my attention was directed to some curious facts about 'Persian Berries,' the fruits of certain species of *Rhamnus* used in dyeing. It had long been known that a beautiful golden yellow solution can be obtained by simply macerating these fruits in water, and various researches on the part of chemists had demonstrated that the dye is obtained chiefly if not entirely from the husks (pericarps); at the same time dyers and others knew that the crushed berries yield a satisfactory colouring matter, whereas the pericarps alone do not. For some reason it is necessary to employ the inner parts of the fruits as well as the pericarps; these inner parts of course include the seed when the berries are crushed whole, as is the usual practice. Various species of *Rhamnus*—*R. Amygdalina*, *R. infectorius* (*R. oleoïdes*[1]), *R. saxatilis*, &c.—are employed as 'Persian Berries,' and although the following remarks apply particularly to *R. infectorius*, there are reasons for believing that they may apply generally to other species also.

[1] Mr. Thiselton Dyer informs me that this is a synonym of *R. Amygdalina*.

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In 1842 Fleury examined a yellow dye got from the fruits of a *Rhamnus*; a little later Kane obtained a body which was called *chrysorhamnin* from unripe fruits, and another from ripe fruits which he named *xanthorhamnin*. Gellatly in 1851 gave a complex formula (C_{48} H_{56} O_{28}) to this xanthorhamnin, and stated that dilute sulphuric acid causes it to break up into a body called *rhamnetin* and grape-sugar—in other words, that xanthorhamnin is a glucoside. This was confirmed subsequently.

In 1879 Liebermann and Hörmann, employing *R. infectorius*, confirmed the foregoing, and found that no dye (or the merest traces) is obtained from the seeds, but that it exists in the husks (pericarps). They got the glucoside xanthorhamnin, which is soluble in water and alcohol, but not in ether, benzole or chloroform, and assigned to it the formula C_{48} H_{66} O_{29}. It is soluble in alkalies; ferric chloride turned the solution brown. Sulphuric acid causes it to break up into glucose and rhamnetin.

They also found that the xanthorhamnin breaks up under the action of some ferment in the fruit, the products of this reaction being a colouring body, *rhamnin* and glucose.

This was practically the position of our knowledge, when my attention was directed to an experiment performed by Mr. T. E. Lightfoot, of Accrington, a gentleman interested in dyeing, and who was then investigating the qualities of the different yellow dyes obtainable from 'Persian Berries.' Mr. Lightfoot has informed me by letter that he found that a decoction of the uninjured berries yielded a poorer colouring liquor than one obtained from the ground or crushed 'berries.' He then took some of the fruits, and split them, separating the outer shells—the chief part of the pericarp—from the 'kernels'; these 'kernels' are the seeds, and they are covered by a thin hard covering which, as will be seen shortly, is the endocarp.

A weighed quantity of the outer pericarps was then used for making a decoction, and a piece of cloth dyed with the liquor; in another vessel the same weight of 'shells' (pericarp) was used, but a few of the 'kernels' (seeds) added.

In the second case the colouring matter was a brilliant golden yellow, whereas the former gave but a poor lemon-yellow dye.

The outer pericarps were next digested in water at 45°C. for about one hour, and the clear yellow filtered liquor was placed aside with a few 'kernels' (seeds) added; in thirty minutes or so a light yellow powder fell to the bottom, C O_2 being given off meanwhile.

'Kernels' were then split up into four parts, and these parts kept separate. (1) The shell or husk of the 'kernel' (i. e. the endocarp); (2) A greyish white matter (i. e. endosperm); (3) the infolded rims of the seed proper; (4) a yellow substance—the embryo—inside the seed.

To clear decoctions of the outer pericarps Mr. Lightfoot then added the different parts of the dissected kernels, and found that in every case the yellow powder fell after a time, but more quickly where the rims of the seed were added. The action was destroyed by boiling.

The obvious explanation of the above experiments is that a ferment, localised in the 'kernels' (seeds), acts on the yellow substance dissolved from the pericarps.

Having obtained some fruits of *Rhamnus infectorius* from Kew, I set to work to investigate the matter independently.

I found that if the whole fruits are steeped in water, and kept at 35°C., a quantity of bright yellow substance collects around the swollen mass, and if squeezed out gradually forms a slight precipitate. On breaking these steeped fruits there is abundance of glairy yellow substance inside, not easily washed away. If the fruits are broken up *first*, however, a copious precipitate soon falls; this is yellow, and finely crystalline, and is evidently the rhamnin of the chemists[1]. The filtered liquor after this experiment reduces Fehling's solution, and contains relatively large quantities of glucose.

I then repeated the experiments with the various parts of

[1] Husemann, 'Pflanzen-stoffe,' vol. ii. 1884, p. 889, where the chemical literature is quoted.

the seeds and the endocarp. The chief difficulty here was to obtain perfectly clean portions. It was easy to separate the endocarp; far less easy to separate the testa of the seed from the contents. The large yellow embryos slip out pretty easily. I doubt whether the testa was ever got perfectly free from the nucellus in these earlier trials. A decoction of the freed outer pericarps was then made, and the clear yellow filtered solution placed in test-tubes and treated as follows:—

A: was left alone.
B: testa was added.
C: endocarp was added.
D: endosperm was added.
E: embryo was added.

All the experimental tubes were placed in a warm chamber and kept at 35°C. After half-an-hour I found a copious yellow crystalline precipitate falling in B, and faint traces of a similar precipitate in E. In all the other tubes the liquid was still clear. After eight to twelve hours traces of a precipitate were observable in the other tubes, but it was more than a day after that any noteworthy changes were observable.

Evidently something in the testa (and possibly in the embryo also) acts as a ferment on the yellow glucoside in the pericarp. I repeated the experiment B, but boiled the solution after adding the testa; a coarse cloudy precipitate formed. It became probable later that this was due to the boiling. I again repeated experiment B, with the following modifications. In one case I employed a glycerine-extract of the testa; this was effectual, as before. In another case I used a filtered water-extract of the testa; this was effectual also, but *not so if boiled first.* There seemed to be a soluble ferment in the testa of the seed then, and it is obvious that the results confirmed previous experiments.

It was now time to examine the tissues histologically, and I confined my attention at first to the testa and pericarp. In the cells of the outer pericarp are brittle waxy yellow masses (Fig. 6), which dissolve at once in water, and are evidently masses of the glucoside (xanthorhamnin). The

testa contains thick-walled pitted cells (Figs. 4 and 12) which contain a peculiar finely granular substance, which dissolves at once on adding water and disappears. I was for some time strongly inclined to regard this fine grey powder as the ferment with which we had been experimenting. To test the accuracy of this conclusion I made very thin sections of the dry testa, and placed them directly into solutions of the glucoside from the pericarp; the sections were from all parts of the testa. In less than half-an-hour I found a semi-crystalline precipitate resembling the precipitates of rhamnin obtained in the test-tubes.

I then asked Mr. John Dunlop, who was at that time working in my laboratory at the Owens College, to go over the anatomy and histology of the fruits and seeds of *Rhamnus* with me; this he was good enough to do, and most of the figures in the plates are due to his pencil. We confined our attention to *Rhamnus infectorius* at the time; later on I examined the histology of several other species.

The fruit of *Rhamnus infectorius* is a berry-like drupe (Fig. 1) with a dry waxy outer pericarp, and a thin woody endocarp: within this are three or four erect seeds, which, if separately and completely enclosed in the sclerenchymatous endocarp, might almost be called nutlets (Fig. 2). A horizontal transverse section made equatorially across the drupe reveals the seeds lying loosely in the loculi of the dry fruit, one in each loculus, enclosed in the hard thin endocarp. On splitting this endocarp the seeds fall out, being loose within it; each seed is smooth and shining, brown in colour, and with a longitudinal deep groove on the dorsal side. A transverse section of the seed shows a hard brown testa, doubled in at the dorsal groove (Figs. 3 and 21), the margins of the groove being thickened and harder than the rest. The cavity within the testa is nearly horseshoe-shaped in transverse section, and filled with white endosperm, in which lie the cotyledons: these are face to face and also horseshoe-shaped in transverse section. Between the endosperm and testa were several rows of broken down and disorganised cells, evidently

the remains of the nucellus. In the dry state all the parts are shrunken, and a large hollow cavity exists on the dorsal side of the endosperm—between it and the testa. The various parts of the fruit were then separated and sections cut so as to exhibit their structure. The outer pericarp is brittle and waxy in texture: their sections, in the dry state, show (Fig. 5) an outer epidermal layer, the external cell-walls of which are strongly cuticularised. With the exception of certain small granules, looking very like plastidia, these cells and those immediately below them have no contents. Immediately below the epidermal layer are four or five rows of hypodermal cells, the outer rows consisting of regular rectangular cells, which in the inner rows become less and less regular and smaller, all however containing small corpuscles near the interior of their walls. These seem to be chlorophyll-corpuscles. Below these cells are larger, thin-walled, parenchymatous cells containing a yellow amorphous substance which completely filled up the cavity of the cell (Figs. 5 and 6).

Water was then added to the dry sections while still under the microscope; the cell-walls, etc. swelled up and the yellow substance in the cells at once dissolved completely, colouring the water yellow and leaving the cavities of the cells empty; the latter were then seen to be thin-walled and parenchymatous (Fig. 5).

To other dry sections under the microscope glycerine was added; they again swelled up and the yellow substance in the parenchymatous cells again dissolved, but not so rapidly as in water. Sections were treated in a similar manner with alcohol, chloroform and ether; the yellow substance dissolved to a very slight extent in alcohol, but was insoluble in chloroform and ether. Thus the yellow substance was not a wax, resin or fat, any of which would probably have dissolved in any of the last three reagents, and not in water or glycerine.

The histology of the endocarp was next made out. Its inner yellow lining was stripped off and examined; it was found to consist of a layer of long thin-walled cells containing a yellow waxy-looking substance, and on adding water the

contents cracked and dissolved like the substance contained in the cells of the outer pericarp, leaving the cell cavities empty and allowing the shape of the cells to be more clearly seen (Figs. 8 and 9). On treating dry sections with glycerine, the yellow substance again dissolved, but more slowly than in water (Fig. 8). This yellow substance was treated with alcohol, chloroform and ether, and acted in the same way as the yellow substance in the parenchyma cells of the outer pericarp. Transverse sections were then cut of this inner husk, and the cells appeared rectangular, the cell-wall being slightly thickened on the exterior surface (Fig. 10).

As regards the rest of the endocarp, thin transverse sections were cut, and were found to consist of a number of rows of hard sclerenchymatous cells, of which the lumina were nearly obliterated, having a very distinct middle lamella between the cells, and with a large number of pits radiating from the lumina to the middle lamella, corresponding to pits radiating from the lumina of contingent cells (Fig. 7).

Thin transverse sections of the testa were then cut, and it was found to consist of a single row of sclerenchymatous cells, with a number of pits radiating from the centre of each lumen, the cell-cavity containing a greyish substance which diffused out of the cell on adding water to the section. The middle lamella of the cell-wall was very well marked (Figs. 11 and 12).

Thin tangential sections of the endosperm were cut, the tissue was found to consist of thin-walled parenchyma, the cells containing protoplasm and various other bodies. On adding water to the sections oily drops separated out in the cells, and exuded at the sides of the sections; the water was then removed and alcohol added, the fatty drops disappeared (Fig. 14). Fresh sections were cut and placed dry in alcohol and examined in glycerine, the fat had dissolved and a number of small grains closely packed were left in the cells. Sections which had been treated with alcohol as before were examined and water added while under the microscope, and these grains dissolved after swelling up. Thus the endosperm contained

fats and grains which there were grounds for thinking were aleurone grains. To confirm this supposition sections were cut and placed in a two per cent. solution of mercuric chloride in absolute alcohol, and were left in it for about forty-eight hours, washed for half a minute in water, stained rapidly with eosin, and mounted in a solution of a neutral salt, potassium acetate being used. On examining the sections a large number of aleurone grains were found in the cells, but on examining the grains no enclosure could be detected within them (Fig. 15). Sections were placed in the alcoholic solution of mercuric chloride as before and left for twenty-four hours, then washed in absolute alcohol, stained rapidly with eosin, washed for half a minute in water, and mounted in a solution of potassium acetate. On examining these sections it was found that the aleurone had been dissolved out by washing in the water, leaving a reticulum of protoplasm in which the aleurone grains had been lying, and showing a well-stained nucleus in each cell (Fig. 16).

Sections of the cotyledons were cut and examined dry, they were a light yellow in colour; on adding water the cells became more distinct, and oily globules separated out, which on removal of the water dissolved in alcohol, chloroform and ether. The sections consisted of about six or seven rows of cells, the outer row on each side being arranged very regularly and with their outer walls slightly thickened. The second row on the one side consisted of columnar cells, while those in the centre were more irregular and larger, and with a number of intercellular spaces between them (Fig. 17).

Sections were treated with the alcoholic solution of mercuric chloride for twenty-four hours, washed for half a minute in water, stained with eosin and mounted in a solution of potassium acetate. On examining the sections it was found that the cells contained a large amount of aleurone, which seemed to be similar to that contained in the endosperm, as no enclosure could be detected within the grains which, on the addition of water, swelled up and dissolved.

The cells of the embryo are thin-walled and parenchyma-

tous, containing protoplasm, aleurone and fats, together with a yellow substance, the nature of which I was unable to make out, but which did not seem to be identical with the yellow substance contained in the outer pericarp and endocarp, and this supposition was strengthened by the action of the various parts on one another, this yellow substance contained in the cotyledons not acting in the same way as the yellow body contained in the pericarp and endocarp.

On cutting sections of the embryo and staining with methyl green a prominent nucleus was seen in each cell (Fig. 18).

The reactions of the whole berry and its various parts were next investigated with the following results. The whole berry was digested in distilled water for about twenty-four hours, the temperature being 30°C.; at the end of this time there was found to be a copious yellow precipitate, which on examination under the microscope was found to consist of clumps of spherical masses aggregated together (Fig. 20). The supernatant fluid was poured off from the precipitate, and to it were added two or three drops of Fehling's solution; after warming, a copious brick-red precipitate of cupric oxide was thrown down showing that a glucose was present in the solution, as no precipitate was obtained when the solution was similarly heated without adding Fehling's solution.

The pericarp was stripped off a number of fruits and digested in distilled water for about twenty-four hours, the temperature being about 30°C. The solution was a pale yellow at the beginning, but after digestion the colour was more pronounced and darker, but no precipitate was obtained. Fehling's solution was added to this liquid and warmed, but no precipitate was thrown down. The pericarp was digested in distilled water for twenty-four hours, at a temperature between 60° and 80°C., and at the end of the time a yellow precipitate was obtained, which was found to consist chiefly of clumps of long needle-shaped crystals (Fig. 19); the liquid filtered off from these gave a precipitate with Fehling's solution. Hence the yellow substance contained in the pericarp, which from its micro-chemical and other reactions, solubility

in water, and insolubility in alcohol, chloroform and ether, must be a glucoside, is split up on heating to about 70° C. into glucose and a semi-crystalline substance.

The endocarp, on treatment in the same way as the pericarp, acted in the same manner; on digestion at 30° C. no precipitate was obtained, and the solution gave no glucose reactions; on digestion at a temperature about 70° C. a crystalline precipitate was again thrown down, and the filtrate from this yields a precipitate on warming with Fehling's solution. Thus the substance in the endocarp acts in the same manner as that in the pericarp, both probably being the same glucoside, but contained in much larger quantities in the pericarp. If the seeds be removed and digested for twenty-four hours, at a temperature of 30° C., an opalescent clear solution is obtained; if the seeds are digested at 70° C. the solution still remains clear, showing that the glucoside is not contained in the seed.

The pericarp was digested in water for twenty-four hours with the endocarp; no change took place.

The pericarp was digested in water for twenty-four hours, at a temperature of 30° C., and the solution filtered off, and added to the solution obtained on digesting the seeds for twenty-four hours at 30° C. After leaving them at 30° C. for half-an-hour a precipitate was obtained, which soon became very copious, and which was found to consist chiefly of needle-shaped crystals, and also of the semi-amorphous masses obtained by digesting the whole fruit (Figs. 19 and 20). The filtrate from this precipitate yielded, on warming with Fehling's solution, a copious precipitate. Thus the yellow glucoside has been again split up into glucose and a crystalline substance, and this time not by heat, therefore it must have been by means of some substance contained in the seed.

If the solution obtained from the seeds is boiled for ten minutes and added to a solution of the pericarp, and allowed to stand for twenty-four hours at a temperature of 30° C., no precipitate is obtained, and the solution does not yield a precipitate on warming with Fehling's solution. Hence the

action of the substance contained in the seed which converted the glucoside into glucose and another substance has been destroyed by heat, and consequently that substance is a ferment.

There was thus a ferment in the seed which broke up the glucoside contained in the pericarp and endocarp. I then proceeded to find out in what part of the seed this ferment was contained. The testa was stripped off a number of seeds and digested at the usual temperature for an hour; a clear solution was obtained, which was added to a solution of the pericarp, obtained by digestion as before, and a copious yellow precipitate was obtained in about twenty minutes. The solution obtained from the testa on digestion was boiled for ten minutes and added to the solution of the pericarp; after twenty-four hours no precipitate was obtained. The filtrate in the first instance yielded a precipitate with Fehling's solution, but none was obtained in the second. Hence the ferment, the action of which was destroyed by boiling, appeared to be contained in the testa.

The rest of the seed was taken and digested, and the solution obtained was added to a solution of the pericarp; in about an hour a copious yellow precipitate was obtained, and the filtrate from this precipitate gave glucose-reactions. Hence the ferment, the action of which was also destroyed by boiling in this case, is also contained in the rest of the seed either in the embryo or endosperm, or both.

The embryos of a number of seeds were dissected out and digested as usual, and the solution obtained from them was added to the solution of the pericarp; after a few hours a yellow precipitate was again obtained, the filtrate from which yielded a precipitate on warming with Fehling's solution. Hence the ferment appeared to be contained in the embryo.

The endosperm was removed from a number of seeds and digested for twenty-four hours, at a temperature of 25° C., and to the solution obtained was added the solution obtained by digesting the testa, and the mixture was allowed to stand for twenty-four hours at the same temperature; at the end of the time no precipitate was obtained, and the solution gave no

glucose-reaction, hence the ferment was not contained in the endosperm.

On cutting sections it was noticed that there was a large quantity of a yellow substance in the cells, which however differed considerably from the glucoside of the pericarp in its micro-chemical reactions. In order to see if it was different from the glucoside the embryos of a number of seeds were digested as usual, and to this solution was added a solution of the testa; on allowing to stand for twenty-four hours no precipitate was obtained, and it was therefore concluded that the yellow substance of the embryo was not the same as the glucoside of the pericarp.

Thus in the pericarp there is contained a glucoside, which is split up by a ferment contained somewhere in or near the testa and embryo into glucose and a crystalline substance.

It seemed to be proved from the foregoing observations that the ferment which decomposes the glucoside (xantho-rhamnin), contained in the cells of the pericarp, is localised in the testa of the seed; whether any traces existed in other parts was not proved. On adding water to the intact fruits the soluble ferment passes out and acts on the dissolved glucoside from the pericarp, breaking it up into rhamnin and glucose. If this occurs the precipitate will be withheld chiefly inside the fruits, thus explaining why the dyers should crush their fruits, since it is the insoluble semi-crystalline precipitate which they want.

Before proceeding to show where the ferment really is—in the raphe, a discovery which I only made some time after—it should be stated that the above observations were unavoidably put aside owing to the pressure of new duties. I had, however, made some observations which led to the suspicion that the ferment is even more localised than it had so far been shown to be.

Series I.

The following experiments were made with 'Persian berries' —the fruits of *Rhamnus infectorius*—obtained from Kew.

A. Six of the fruits were placed intact in cold distilled water, in a labelled test-tube, and the whole kept at 15° to 16°C. in a hot-house for twenty-four hours, perfectly at rest.

The fruits all floated. In the course of some hours a yellow cloud was observed round the fruits. After twenty hours a copious yellow precipitate had fallen to the bottom of the pale, lemon-coloured liquor: a similar precipitate was sticking to the outsides of the fruits.

B. Six of the fruits were slightly *crushed*, and treated in all respects exactly as in *A*.

The cold water at once turned pale lemon colour, diffusion streaks falling from the floating pieces of fruit as the water dissolved the yellow glucoside from the pericarps: in ten to fifteen minutes the solution was of an intense, clear lemon colour. In two hours a bright golden-yellow precipitate was falling to the bottom, and in three hours there was a copious precipitate[1].

C. Six of the fruits were placed intact in a tube as before, but *boiled* for ten minutes: then treated exactly as in *A*.

A deep golden, clear liquor at once resulted: the fruits fell to the bottom of the tube, leaving the perfectly clear solution above. There was no precipitate—not even a cloudiness—after twenty-four hours.

D. Six fruits were crushed, and then treated exactly as *C*.

The result was the same—no precipitate was formed in twenty-four hours owing to the ferment being destroyed by the boiling.

E. Six of the fruits were dissected, and the outer pericarps alone taken and treated exactly as in *A*.

A pale yellow solution was at once produced, and slowly became more and more intense as the xanthorhamnin was dissolved from the cells. The liquor remained perfectly clear even after twenty-four hours[2].

[1] This precipitate was rhamnin, slowly forming in the quiescent liquor, as the ferment acted on the glucoside, xanthorhamnin: obviously the quicker action here was due to the fruits having been crushed.

[2] And longer, for there was no turbidity to be seen on the following day again.

F. The hard endocarps of the above six fruits (*E*) were taken separately and treated exactly as in *E*.

The endocarps floated, and slowly tinged the water pale yellow[1]. The pale yellow solution remained perfectly clear even after twenty-four hours.

G. The seeds from the above six fruits (there were eleven good large ones) were separately treated with cold water &c., exactly as in *E*.

All floated. The water remained perfectly transparent and colourless even after twenty-four hours. After thirty-six hours I noted a slight turbidity: this increased rapidly, and was found to be due to *Bacteria*.

H. Six fruits were dissected, and the outer pericarps alone taken, and boiled for five minutes in distilled water, then treated exactly as before.

All fell to the bottom, and yielded the same deep clear golden liquor as in *C*. No precipitate or other change resulted, even after fifty hours.

I. The hard endocarps of the above (*H*) six fruits were treated exactly as before, except that they were kept in glycerine (1 vol. glycerine to 1 vol. water). All floated. No change occurred beyond a yellow tinge, as the glycerine dissolved the colouring matter.

K. The twelve best seeds obtained from the six berries dissected (*H*) were placed in a test-tube and glycerine (as in *I*) added. All floated. No change occurred even after fifty hours.

In the next series of experiments, I confirmed more exactly what is already to be seen from a comparison of the above. No precipitate appeared in *C* and *D* because the ferment was destroyed by the boiling: the absence of a precipitate in the case of *E* and *H* is simply due to the absence of the ferment—which exists in *G* and *K*, apart from the fermentable glucoside. *F* and *I* also contain no ferment.

[1] From the layer of cells lining their insides.

Series II.

L. I now took the solution *A*, and filtered off the precipitate and fruits from the clear golden liquor.

The liquor contained large quantities of glucose on testing with Fehling's solution. Part of the liquor was allowed to stand, and in twenty-four hours a slight further precipitate had formed. (N.B. This did not occur with the liquor from *C* (q.v.) which had been boiled. Some ferment had no doubt passed through the filter.) The golden semi-amorphous precipitate was then examined microscopically,[1] &c.

M. The contents of test-tube *B* were treated in the same way, and the results were practically the same in all respects.

N. The test-tube *C* was taken, and the clear sherry-gold liquor filtered off from the fruits, and examined. The liquor was divided into three parts, in tubes marked *N* 1, *N* 2, *N* 3.

(1) *N* 1. Added a few drops of solution *G* (i.e. cold-water extract of seeds). A copious precipitate of rhamnin was formed during the night.

(2) *N* 2. Added a few drops of solution *G*, and *boiled two minutes.* It remained perfectly clear for two days.

(3) *N* 3. Added nothing. The solution was perfectly clear next day.

The explanation of this is that the solution of xantho-rhamnin in *C* was incapable of breaking up (*N* 3) spontaneously; but is rapidly decomposed when a solution (*N* 1) containing the proper ferment is added to it. The action does not take place, however, if the ferment is destroyed by boiling for two minutes (*N* 2).

O. I then took the test-tube *D*, and filtered off the clear deep sherry-coloured liquor.

Some was tested for glucose, but gave none. The rest I divided into two parts, in test-tubes marked *O* 1 and *O* 2 respectively.

[1] The results are embodied in the text.

O 1. To this was added some of the solution *F* (i.e. aqueous extract of endocarps[1]. No precipitate was formed—the solution remained perfectly clear for two days.

O 2. To this were added a few drops of solution *K* (i.e. glycerine extract of seeds). Remained perfectly clear for several hours, but a precipitate fell during the night[2].

P. I then took the test-tube *E*, and filtered the clear yellow liquor off from the pericarps, and divided the solution into three parts, marking the test-tubes *P* 1, *P* 2, *P* 3 respectively.

(1) *P* 1. Added a few drops of the solution *G* (aqueous extract of seeds): an *abundant* precipitate fell during the night.

(2) *P* 2. Added a few drops of solution *I* (glycerine extract of endocarp): no trace of turbidity or precipitate was observable next day—nor after forty hours.

(3) *P* 3. Added a few drops of solution *K* (i.e. glycerine extract of seeds): slight precipitate fell during the night and increased slowly during the following day.

Series III.

I now prepared a series of solutions of different parts of the seeds, each in a labelled test-tube, and placed them in the hot-house for the night.

No. *a*. The testa only of the seeds, with distilled water.

(The liquid remained quite transparent and clear for two days, and then became turbid as *Bacteria* developed.)

No. *b*. The testa only used, and pure glycerine poured on it.

A perfectly clear, colourless solution resulted.

No. *c*. Endosperm and embryo cleaned of testa, taken and extracted with cold distilled water.

A limpid colourless extract resulted. (Traces of turbidity next day, and after forty-eight hours it was dirty-white and cloudy[3].)

[1] I only repeated this experiment here to make the series more complete: no trace of the ferment occurs in the endocarp.

[2] The precipitate formed much more slowly than when the aqueous extract of the seeds were used.

[3] Bacteria, &c.

No. *d*. Endosperm and embryo—as clean as possible, but cannot be sure of their purity.

(In two days turbid and dirty-white from Bacteria, &c.)

No. *e*. A few drops of No. *a* (i. e. aqueous extract of testa) were added to the clear solution *H* (i. e. boiled aqueous extract of pericarps).

The No. *a* solution had been prepared 1½ hours; the *H* solution two days; the transparent yellow solution yielded a dense yellow precipitate during the next hour. Other portions of *H* remained clear for two days [1].

No. *f*. To another sample of the yellow solution *H*, I added a few drops of No. *c* (i. e. aqueous extract of clean endosperm, 1½ hours old).

No precipitate was formed.

No. *g*. Another sample of *H* was allowed to stand for forty-eight hours.

No precipitate formed.

The following series of experiments were made with parts of seeds which had been kept dry for two years:—

Series IV.

No. 1. Glycerine (pure) extract of embryos only.

Slowly formed a yellow clear solution which remained clear for days.

No. 2. Aqueous extract of embryos only.

At once formed a yellowish solution. It remained clear for twenty-four hours, but flocculent clouds appeared in two days [2].

No. 3. Aqueous extract of the thickened inturned margins of the seed.

At the time I thought this consisted of the testa only, but pieces of the raphe were also attached.

[1] This apparently again shows that the ferment is in the testa; but see below.

[2] Bacteria.

A slightly opalescent colourless solution. Turbid in two days.

No. 4. Aqueous extract of endosperm, cleared from embryo and testa.

A colourless solution; slightly opalescent in two days.

No. 5. Aqueous extract of pieces of testa taken from the *sides* of the seed.

(The testa can be more easily removed *clean* from the sides of the seed than elsewhere.)

Clear colourless solution.

No. 6. To a fresh solution of boiled pericarps, prepared and filtered as before, I added a few drops of solution No. 1 (i. e. glycerine extract of embryos) which had stood for 1½ hours in hot-house.

No results in two days.

No. 7. To another portion of the pericarp extract added a few drops of solution No. 2 (i. e. aqueous extract of embryos), same age, &c.

No results in two days.

No. 8. To a third portion of pericarp extract added a few drops of solution No. 3 (i. e. aqueous extract of thick margins of testa—and raphe, as I discovered later).

A dense yellow precipitate began to fall in half an hour, and became more and more abundant during the night.

No. 9. To a fourth portion of extract of pericarp added a few drops of solution No. 4 (i. e. extract of endosperm in water): same conditions.

No results at all.

No. 10. To a fifth portion of the same pericarp extract added a few drops of solution No. 5 (i. e. aqueous extract of *sides* of testa only).

Next day were very doubtful traces of turbidity, but not the slightest precipitate [1].

[1] Nor did any precipitate fall in the next twenty-four hours.

No. 11. To a sixth portion of the pericarp solution added nothing.

It remained unaltered and clear for two days.

It had now become clear that (1) the ferment is contained somewhere in the seed; (2) it is confined broadly to the testa or outer coat of the seed; and (3) moreover is localised—situated somewhere in *or near* the thickened margins where the testa is turned in. (4) The ferment is not destroyed by keeping for two years.

I now repeated many of the experiments given above, with the seeds and fruits of the same species (*Rhamnus infectorius*) but from another source, and which had not been kept so long. I first dissected twenty-four seeds, under the simple microscope, separating the thickened margins of the testa (where it is turned in) from the rest of the seeds—i. e. the testa of the sides, the endosperm, and the embryos.

Series V.

γ. An aqueous extract of the thickened inturned margins of twelve seeds.

Yielded a perfectly clear colourless liquid.

δ. Aqueous solution of the rest of the twelve seeds (i. e. the endosperm, embryos, and the testa from the sides of the seeds).

Yielded a clear, colourless liquid [1].

η. A glycerine extract of the thick inturned margins only of twelve seeds.

Perfectly clear extract.

θ. A glycerine extract of the rest of the seeds.

Clear solution.

I then added a few drops of each of these extracts to a series of test-tubes containing fresh boiled solutions of the glucoside (xanthorhamnin) obtained from the pericarps.

[1] Faint tinge of yellow.

The tubes were labelled, and treated as before. We may call this

SERIES VI.

A*. A few drops of the extract γ (i. e. aqueous extract of margins of testa) added.

A dense cloud formed in ten minutes, and an abundant golden precipitate was falling in fifteen minutes.

(N.B. All the following tubes were still perfectly clear.)

B*. A few drops of solution δ were added (this was an extract of all the rest of the seed).

A precipitate was *slowly* forming three hours later: this slowly increased, and was abundant next day[1].

C*. A few drops of solution η (i. e. glycerine extract of thick margins only) were added.

A slight precipitate had fallen in two hours, and increased soon after till it was as copious as in A*.

D*. A few drops of θ were added (i. e. glycerine extract of all the rest of the seed).

This remained perfectly clear for many hours, but a precipitate fell next day.

Here was an apparent contradiction of some of my previous statements and conclusions, and it became necessary to see why the precipitate fell in the tubes containing the extracts of other parts of the seeds than the thick margins. Before explaining this, however, I will give one more series of experiments.

SERIES VII.

E*. To a fresh solution from boiled pericarps added the thickened inturned margins of six seeds, dissected away as clean as possible under the simple microscope.

A precipitate began to form after three hours, and this was abundant next day.

[1] I shall show below that this was contaminated, and how.

F*. To another portion of the pericarp solution I added the cleaned sides of the testa only of the same six seeds.

The solution remained clear for two days.

G*. To another portion of the pericarp solution added the dissected out endosperm and embryos from the six seeds.

The solution remained perfectly clear for two days.

H*. Another portion was allowed to stand untouched, and it remained clear for several days.

Here then we see that no ferment was present in the *sides of the testa*, the endosperm, or the embryos: how was it, then, that the ferment existed in the solutions B* and D* of Series VI? This question is best answered after examining Fig. 21. It will be noticed that the raphe (adherent funicle) of the seed runs in the dorsal depression of the seed, and that *it is adherent to the testa proper* all along the sides of the groove of the seed. In other words, when the testa begins to harden, the lignification does not extend to the raphe, but is confined to a layer of cells—the outer integument of the ovule—which runs inside the raphe. When the fruit and seeds are dried, the thin-walled parenchyma of the raphe shrivels up, and it is not very easy to detect it when dissecting the seed, unless its presence has first been pointed out. As shown in Figs. 21 and 22, however, this raphe is organically continuous with the testa at the groove, and it is almost impossible to clear it away from the hard testa at this part. As soon as I had got at the fact that it is the *raphe* which contains the ferment (and this follows with certainty from the experiments below) all the errors were cleared up. First, however, I had perhaps better give the results.

It had struck me several times that the ferment was very energetic, because such a mere trace of the solution containing it caused such copious precipitates to form. This being the case, I prepared a series of moist chambers, such as are used for growing Fungi, &c. in beneath the microscope. I then cut fairly thin transverse sections across the whole seed—dry and unaltered—as shown in Figs. 2, 3, 21. It was now very

easy to separate very tiny bits of the various parts, testa, endosperm, embryos, and *raphe*. I then did this with needles. I first separated a minute piece of the raphe, and placed it in a tiny drop of fresh extract of pericarps, hanging from the cover slip over the moist cell: then I heated the needle points and separated a bit of the outside testa, and placed it in a similar hanging drop; and so on with endosperm and embryo.

The result was startling. A copious precipitate had formed in the drop containing the bit of raphe, before I had finished preparing a second specimen—i.e. in less than five minutes. Summing up the results of numerous repetitions of these experiments in drops, I find that the slightest piece of the raphe causes decomposition of the glucoside xanthorhamnin in two or three minutes: under the microscope a cloud of minute black dots (black because so small?) arise in the previously clear yellow solution, and grow under the eye of the observer into the typical semi-crystalline yellow masses of rhamnetin. The whole process occupies a few minutes, and I have now demonstrated it several times to others.

We now see why some of my previous experiments yielded ambiguous results. Starting from the fact that the slightest trace of the ferment—and therefore the merest little piece of raphe containing it—will start the decomposition, it is easily seen that while bits of the thickened margins of the inturned testa (which always have adherent to them cells of the raphe) produced the decomposition, the pieces of outside testa from other parts of the seed could easily be got clean, and no decomposition followed. In cases where I neglected the presence of the shrivelled film of raphe adhering to the testa lining the groove, as in No. 8 (Series IV), B* (Series VI), D* (Series VI), there were portions of the raphe adhering to some of the pieces dissected out, hence the apparently contradictory results.

As a final illustration of the power of the ferment, I may quote the following experiment. I prepared five cubic centimetres of the solution from the pericarp, and placed the raphe (carefully separated) of *one* seed in it: the raphe floated,

partly because it had air clinging to its dry cells. In ten minutes the floating raphe was thickly covered with the golden yellow precipitate of rhamnetin. In twenty minutes the clouds of precipitate were falling in the tube: in less than an hour there was a precipitate at the bottom of the test-tube, which measured $1\frac{1}{2}$ mm. in depth, and which was many times larger than the raphe.

Examination of the raphe shows that it consists of a slender vascular bundle running up the groove in the seed, which is dorsal, and cellular prolongations like wings from its sides: these two wing-like expansions of thin-walled parenchymatous cells line closely the sides of the groove (Fig. 21). In fact we may regard the vascular bundle of the raphe as the line round which the folding occurs which gives the seed its grooved appearance, and therefore its horseshoe-shaped transverse section. When the folding of the seed occurs, it doubles back with it the two wings of the raphe, and the margins of these wings end in the thickened ridges of the testa, as shown in the figures.

I also examined the contents of the cells of the raphe. When a thin section of the dry raphe is placed in strong glycerine the cells are seen to contain a brilliant, oily-looking, colourless substance which does not fill up the cavity but is driven aside by large vacuole-like chambers in which a few brilliant granules may be observed. If placed in very dilute solutions of caustic potash, the cells and their contents at once swell up, and the oily-looking matrix dissolves almost entirely, but not quite; drops of clear oil-like substance flow together, and escape.

When placed directly in water, the colourless oil-like matrix froths up in a most remarkable manner, and oily-looking drops escape; these drops are vacuolated, however, and something seems to be dissolved from them also. In one per cent. Osmic acid solution, the vacuolated oily masses slowly turn brown. Absolute alcohol dissolves a large proportion of their substance, but not all.

I have examined the fruits and seeds of four other species

of *Rhamnus*—viz. *R. tinctorius*, W. & K., *R. Carolinianus*, Walter, *R. Wicklius*, and *R. catharticus*, L.—but have found no trace of the ferment in any of them. This being the case, I shall sum up the chief points respecting these fruits, &c. in a very few words.

All the fruits were ripe, and the pericarps dark purple and soft, yielding violet and purple brown solutions when extracted with water. The colours of the solutions soon change and become browner or redder, and paler. The addition of a few drops of ammonia in all cases turns the purple or brown solutions green, apparently due to the formation of a pigment like 'sap-green.' These brown or purple solutions also contain glucose, precipitating abundantly from Fehling's solution.

In no case was I able to obtain any ferment action when the seeds of any species were added to the extract from the pericarps of its own or other fruits. The seeds did not precipitate rhamnetin in the solutions of xanthorhamnin obtained from the pericarps of *Rhamnus infectorius;* nor did the seeds of the latter species cause any precipitate to form in the liquors obtained from the pericarps of the above four species. It seems safe to conclude, therefore, that no ferment is present in the seeds of these four species, at any rate when the fruits are ripe.

This raises the question, is the ferment present in the seeds of *R. infectorius* when fully ripe? I cannot yet answer this question, because I do not know whether the fruits used in the trade as 'Persian Berries' are ripe when gathered. Probably they are not, but are cured while yet not quite mature.

If the precipitate of rhamnin is chiefly withheld inside the fruits, this explains why the dyers obtain poorer results by this method, since the crystalline precipitate of rhamnin is what they want.

The soluble ferment was regarded as probably existing in well-protected sclerenchymatous but pitted cells of the testa, as a fine granulated mass; but I have now demonstrated that it exists in the parenchymatous cells of the raphe.

We have now to enquire as to the biological significance of these matters. I have germinated the seeds of *Rhamnus infectorius* once or twice, but have frequently failed to make the seedlings grow at all.

So far it has been only from whole fruits that seedlings were obtained: all the specimens of shelled naked seeds have failed. In the successful cases the pericarps swell, and become yellow and slimy, the soil around being dyed with the colouring matter, and fungi seem particularly apt to appear on the rotting pericarps. It seems an obvious suggestion that this is due to the glucose. But it is also a suggestion worth further investigation that the glucose is of use to the young embryo, and I am strongly inclined to the belief that this is the case, and that the cause of failure with naked seeds lies partly in this. At the same time the matter needs further investigation.

Another point is--do birds or other animals eat the fruits? I find them distinctly bitter and resinous to the taste at first, and they are known to be purgative. It seems not unlikely then that they are not eaten by animals, though they may be carried and broken by them.

My idea that the glucoside stored in the pericarp is for the benefit of the young plant is not without support from analogy, and it is well known that the amygdalin of bitter almonds is contained in certain cells of the seed, the ferment (emulsin) in other cells: so also with mustard seeds—the ferment (myrosin) which breaks up potassium myronate into glucose and other bodies exists apart in the seed.

It is, however, a peculiarity that in this case the glucoside should be in the pericarp and the ferment in the raphe of the seed, a phenomenon which is again suggestive in view of our growing conviction that many glucosides, hitherto regarded as more or less waste products, are really not excluded from the constructive metabolism of the plant.

EXPLANATION OF FIGURES IN PLATES I. & II.

1. Whole fruit of *Rhamnus infectorius*. *a* with three seeds. *b* with four seeds.

2. Transverse section across the whole dried fruit. *p.* pericarp. *enp.* endocarp. *t.* testa. *enm.* endosperm. *em.* embryo.

3. Transverse section of one seed. *t.* testa. *enm.* endosperm. *em.* embryo.

4. Transverse section of part of the seed in water. *t.* testa. *enm.* endosperm. *cot.* cotyledons. Between the testa and endosperm a layer of broken down cells. Zeiss D/2 reduced one half.

5. Transverse section of the pericarp, examined in water. Zeiss D/2.

6. Transverse section of the pericarp, examined fresh in glycerine. The yellow substance (*a*) in the cells is already dissolving. D/2.

7. Transverse section of endocarp, examined in glycerine. Typical sclerenchymatous cells, with the lumina nearly obliterated. F/2.

8. Inner surface of the endocarp shaved off, examined fresh in glycerine. The yellow substance in the cells already partially dissolved. D/2.

9. Inner surface of the endocarp, after being left some time in glycerine. The yellow substance has completely dissolved. D/2.

10. Transverse section of the inner layer of the endocarp, the cells seen in (8) and (9). D/2.

11. Very thin transverse section of the testa in glycerine. Hard sclerenchymatous cells, with a number of pits in the walls. D/2 reduced one half.

12. Tangential section of the testa in glycerine, showing sclerenchymatous cells with pitted walls and containing a granular substance of greyish colour. D/2.

13. Tangential section of endosperm in glycerine, containing fat and aleurone. D/2.

14. Tangential section of endosperm, in glycerine after water and after the fat has been dissolved out in absolute alcohol. D/2.

15. Tangential section of endosperm, which had remained 72 hours in a 2 % solution of mercuric chloride in absolute alcohol, washed ½ minute in water, stained with eosin, and mounted in potassium acetate. The cells contain aleurone in which no enclosure could be detected. The aleurone dissolved in water. F/2.

16. Tangential section of endosperm, 24 hours in the mercuric chloride solution, ½ minute in absolute alcohol, 5 minutes in eosin, and 1 minute in water, mounted in potassium acetate. The aleurone is dissolved out, leaving holes in the matrix, and the nucleus is stained. F/2.

17. Transverse section of one of the cotyledons in glycerine, after removing the fat with absolute alcohol. *f.v.b.* vascular bundle. D/2.

18. Tangential section of a cotyledon in glycerine. *f.v.b.* vascular bundle.

19. Crystalline precipitate, obtained on adding the solution obtained from the pericarp to the solution obtained from the seeds. D/2.

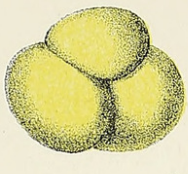
20. Amorphous precipitate obtained on digesting the whole berry. D/2.

21. Transverse section across the middle of a seed, at a level higher than that of Fig. 3. *t.* testa. *enm.* endosperm. *cot.* cotyledons. *r.* funicle (raphe). *m.* thickened margin or ridge of testa. *f.v.b.* vascular bundle of raphe.

22. More highly magnified view of the part of Fig. 21 enclosed in the square. *to.* outer testa. *ti.* inner testa. Other lettering as in Fig. 21.

23. Cells of the raphe in which the ferment is contained.

Fig. 1.



a

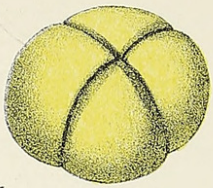


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Fig. 2.

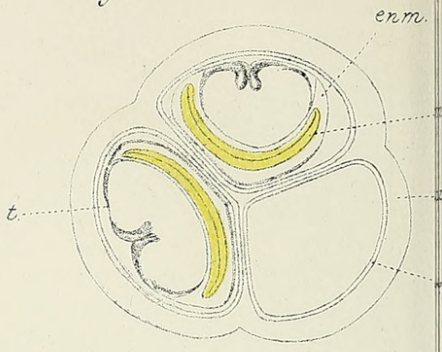
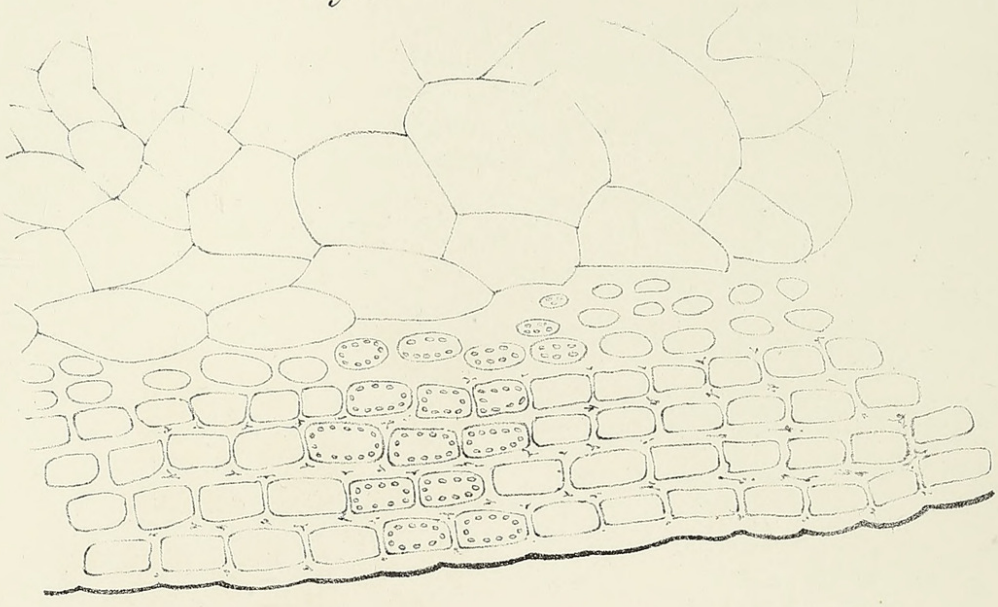


Fig. 5.



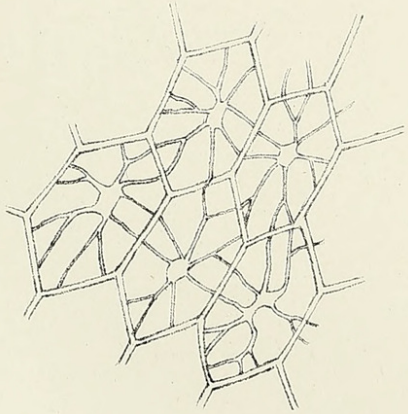


Fig. 7.

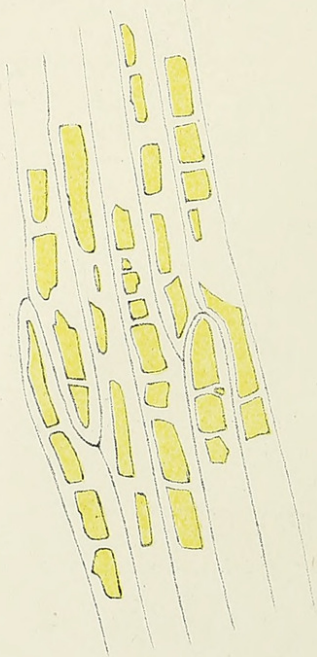


Fig. 8.

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Fig. 3.

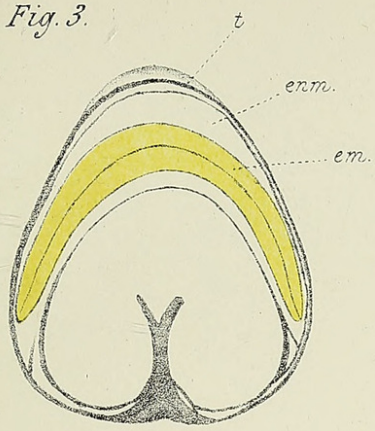
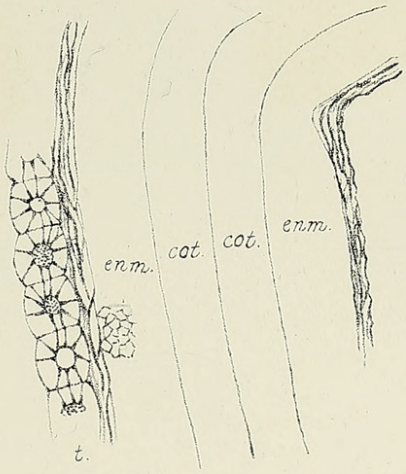


Fig. 4.



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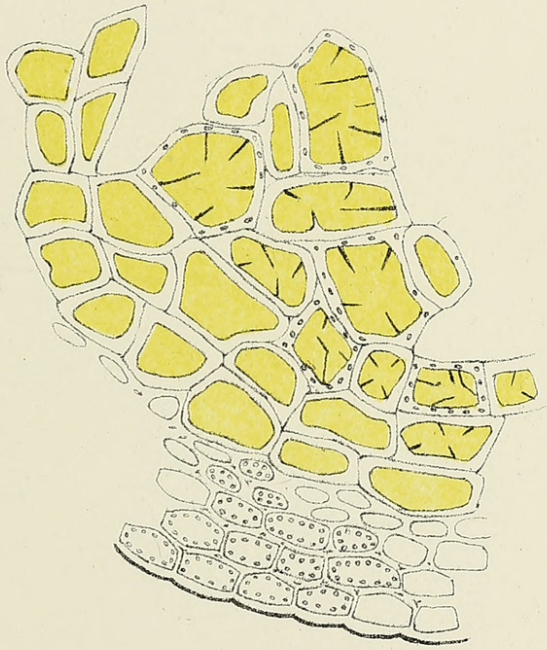

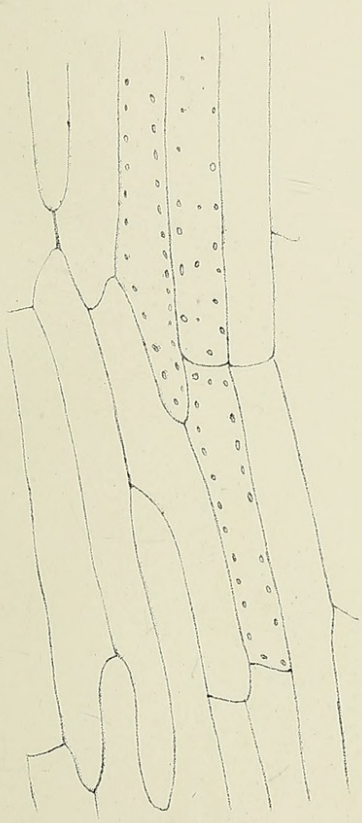

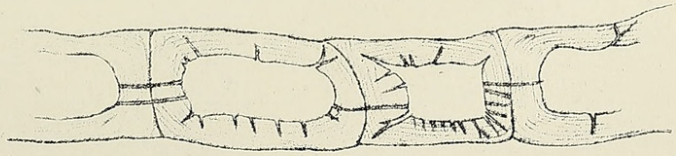

Fig. 10.

Fig. 11.

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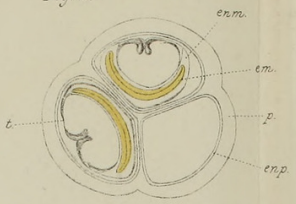
Fig. 1.

Fig. 2.



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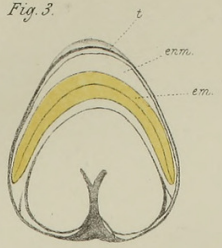

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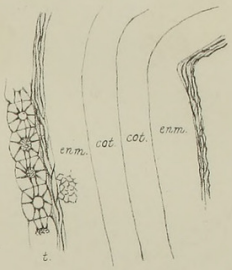

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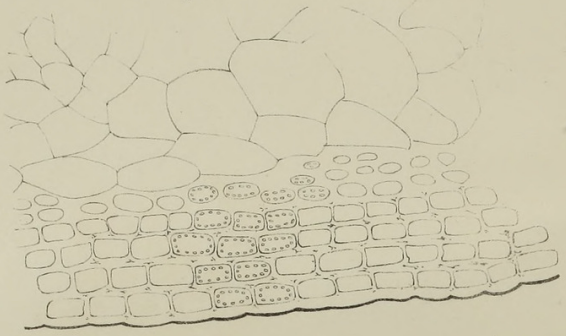

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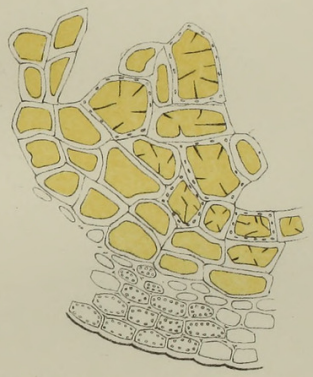

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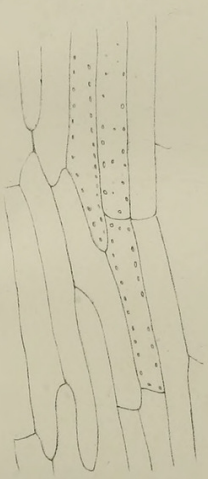

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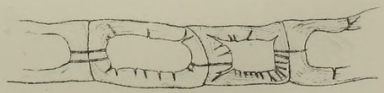

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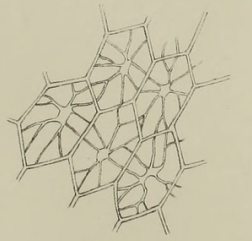

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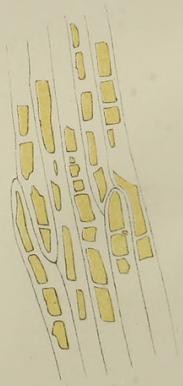

Fig. 8.

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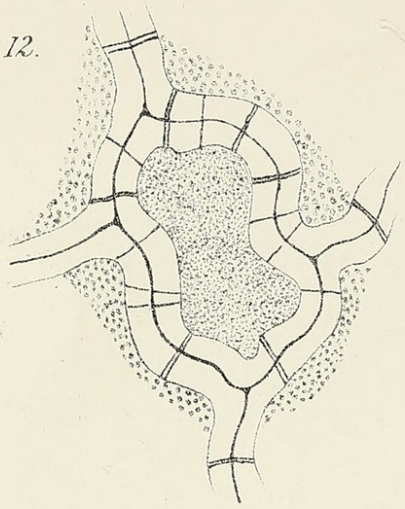
Fig. 12.



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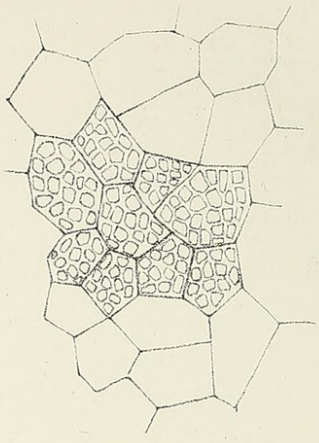

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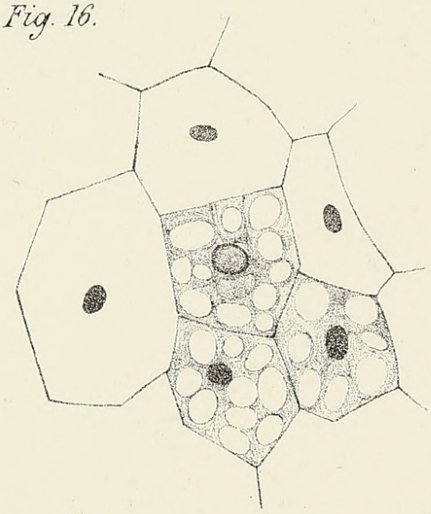

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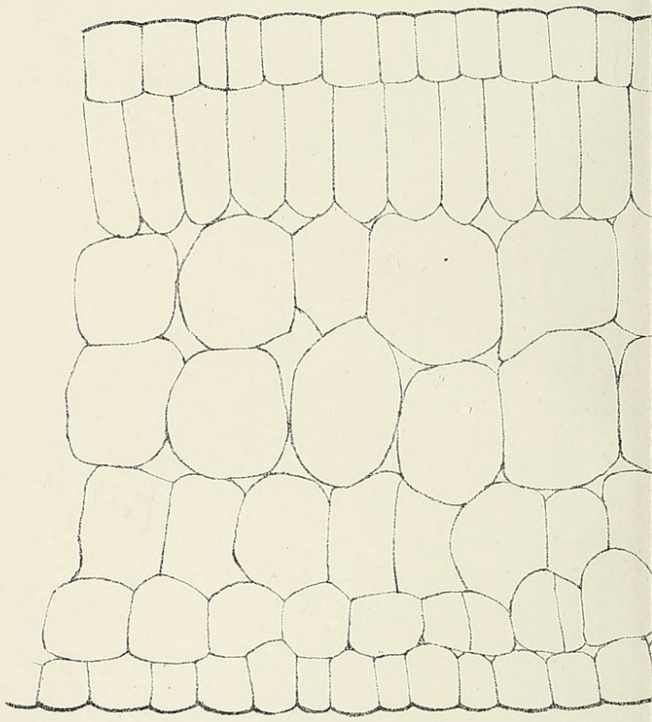

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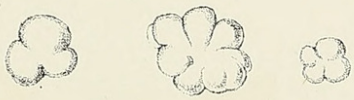
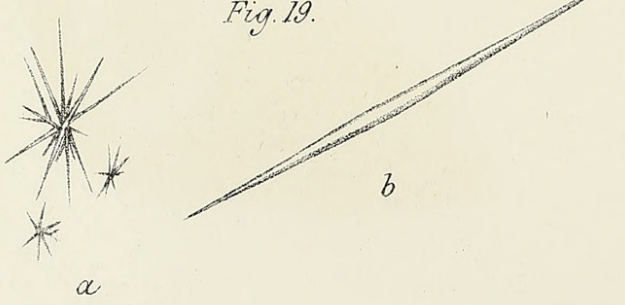


Fig. 19.



a

b

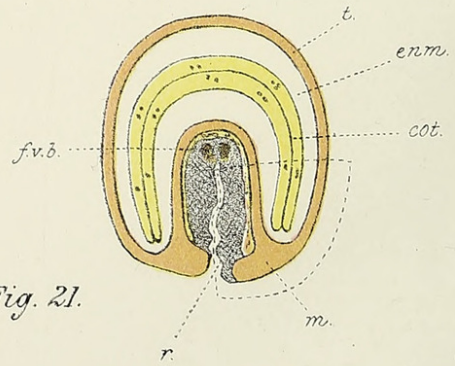


Fig. 21.

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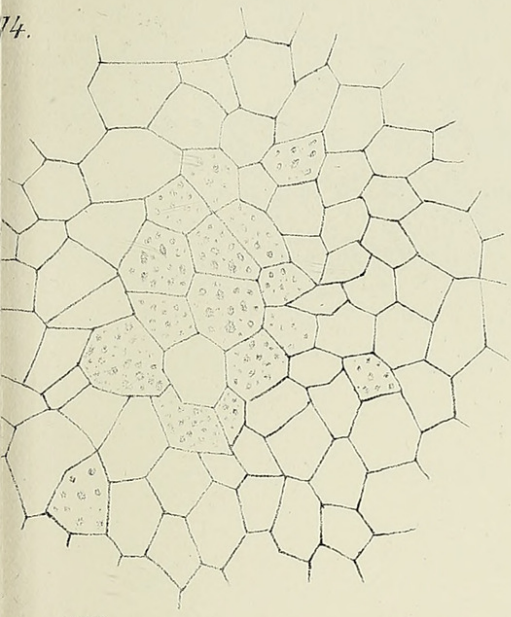
14.



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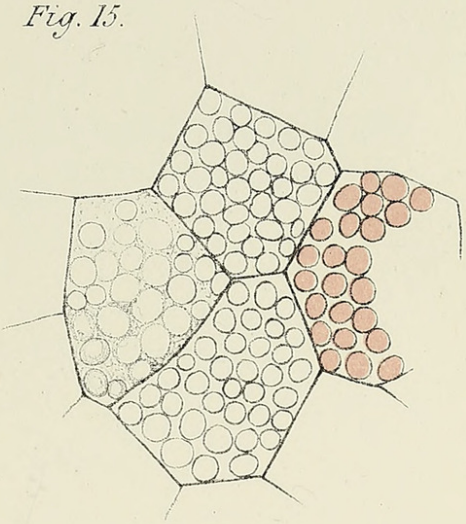

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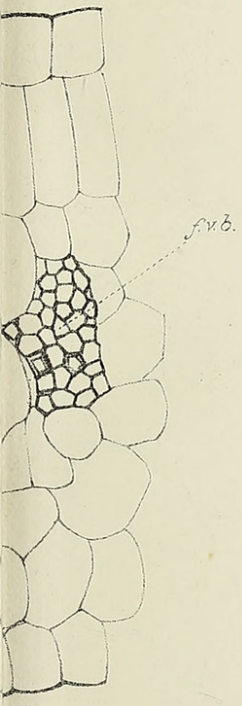

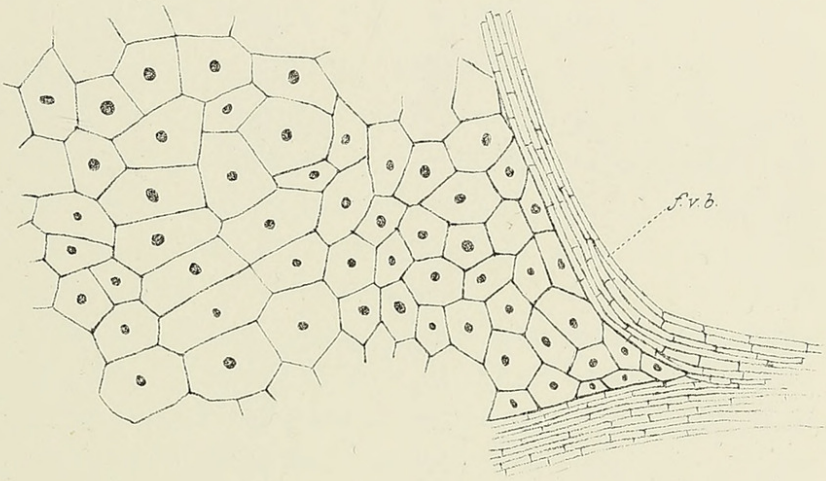

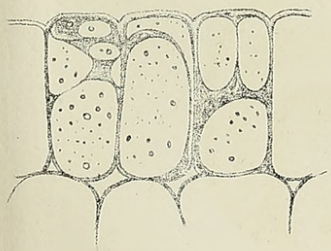

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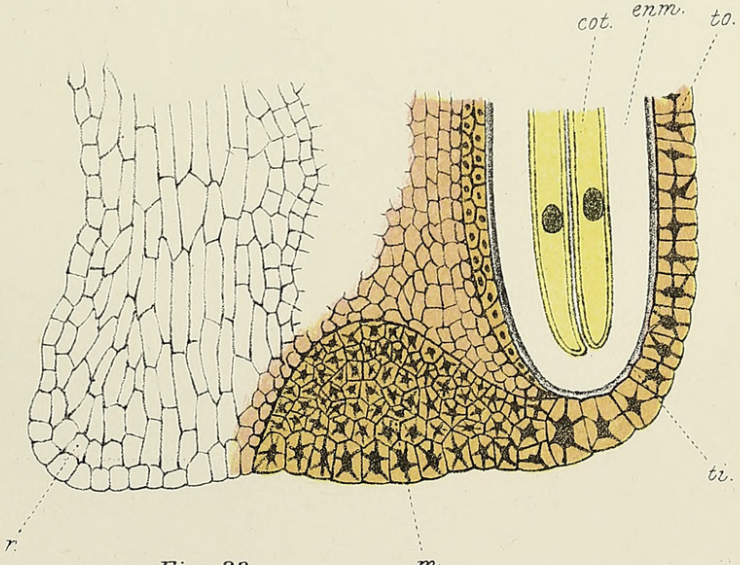

Fig. 22.

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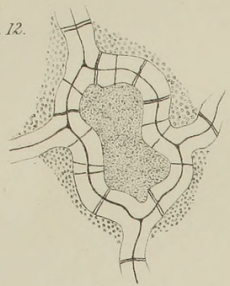
Fig. 12.



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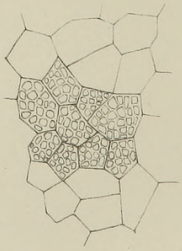

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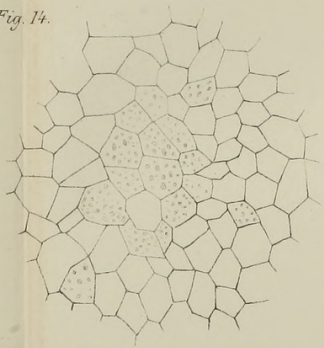

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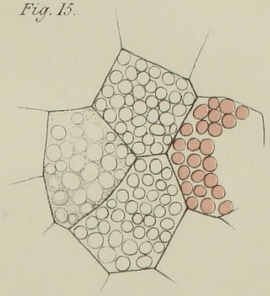

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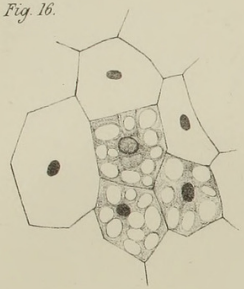

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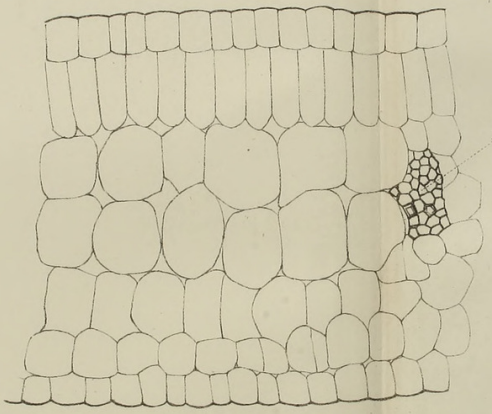

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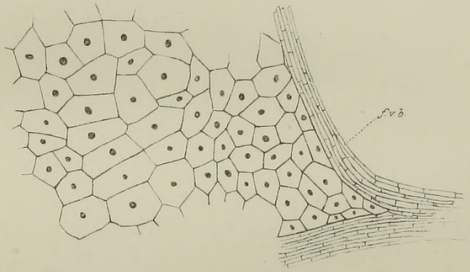

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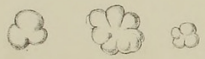

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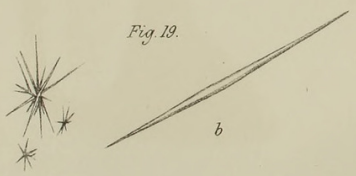

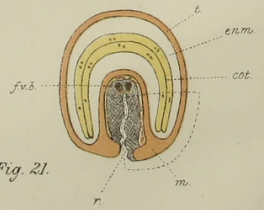

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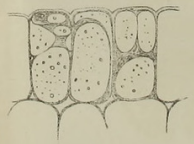

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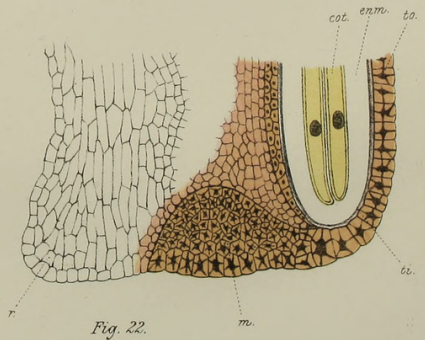

Fig. 22.

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