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A CONTRIBUTION TO THE PHARMACOGNOSY

OF

OFFICIAL STROPHANTHUS SEED

ΒY

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(Read before the British Pharmaceutical Conference in London, July, 1900)

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A CONTRIBUTION TO THE PHARMACOGNOSY OF OFFICIAL STROPHANTHUS SEED.

By PIERRE ÉLIE FELIX PERRÉDÈS,

Pharmaceutical Chemist.

HISTORICAL RÉSUMÉ OF THE "KOMBÉ" DRUG UP TO THE TIME OF ITS INTRODUCTION INTO MEDICAL PRACTICE.

The discovery, in 1861, of the plant yielding the "Kombé" poison is due to Sir John Kirk, and has been described by him in the following words :-- "The source of the poison, namely, Strcphanthus Kombé, was first identified by me. I had long sought for it, but the natives invariably gave me some false plant, until one day at Chibisa's village, on the river Shiré, I saw the 'Kombé,' then new to me as an East African plant (I had known an allied, or perhaps identical, species at Sierra Leone [1858], where it is used as a poison). There climbing on a tall tree it was in pod, and I could get no one to go up and pick specimens. On mounting the tree myself to reach the Kombé pods, the natives, afraid that I might poison myself if I handled the plant roughly or got the juice in a cut or in my mouth, warned me to be careful, and admitted that this was the 'Kombé' or poison plant. In this way the poison was identified, and I brought specimens home to Kew, where they were described."1

The Rev. Horace Waller, who, like Sir John Kirk, was associated with Bishop Mackenzie's missionary expedition of 1861–64, also obtained some pods from a native chief in 1863, and these were brought to England by Sir John Kirk in the same year.

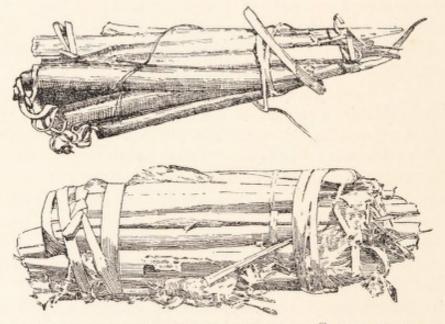
With these pods the first physiological experiments were made, by Dr. Sharpey in 1862–63, and by Messrs. Hilton Fagge, and Stevenson in 1865. These experimenters found that the Kombé arrow poison acted as a cardiac poison.

In 1869 the Rev. Horace Waller presented some specimens of ripe pods to the Materia Medica Museum of the University of Edinburgh, and these, together with additional material obtained

¹ Subsequent investigations have shewn that the "Kombé" seeds of commerce are probably derived from several species of Strophanthus, but the question is too much involved to be discussed here. Suffice it to say that, up to the present, no seeds have been obtained which are positively known to have been gathered from the plant *Strophanthus Kombé* (Oliver).

from Prof. Sharpey and from Mr. John Buchanan, a Scotch missionary, constituted the supply which Dr. Thos. R. Fraser used for his first investigations in 1870. While these investigations were in progress, Dr. Fraser received a poisoned arrow from the same district of Africa as the pods, and, by the physiological action of the poison on it, was enabled to confirm the discovery already made by Sir John Kirk of the source of the arrow poison.

In 1885 Prof. Fraser's communication to the British Medical Association meeting at Cardiff marked the epoch of the adoption of Strophanthus as a remedial agent, and is too well known to require further comment.¹



Two BUNDLES FROM THE FIRST CONSIGNMENT OF STROPHANTHUS WHICH REACHED THIS COUNTRY FOR COMMERCIAL PURPOSES. From original photograph. (These blocks were kindly supplied by Messrs. Burroughs, Wellcome & Co.)

Realising the importance of Prof. Fraser's investigations, and anticipating the influence of these on medical practice, Messrs. Burroughs, Wellcome & Co. sent a trustworthy agent to the Kombé region,² and procured from the native huts the pods stored by the natives themselves for the purposes of arrow poisoning. This supply cost them more than £20 per lb., and constituted the first consignment of Strophanthus introduced for therapeutic purposes.

¹ I am indebted to Prof. Fraser's monograph for all the above information. The full title, etc., of this monograph will be found in the list of literature at the end of this paper, and the reader is referred to the original for an exhaustive account of the history of the drug.

² Chemist and Druggist, March 13, 1886, p. 172.

EXAMINATION OF THE OFFICIAL SEED.

This investigation was undertaken in the first place with the object of determining, as far as possible, what morphological and histological differences exist between various Strophanthus seeds imported from Eastern Africa under the designation of "Kombé" seed. It was shewn by Mr. Holmes (P. J., vol. xxiii., April and May, 1893, pp. 868 and 927) that the albumen and the cotyledons in the "Kombé" seed of commerce, and also in the seeds used by Dr. Thos. R. Fraser in his investigations, shewed striking differences when treated with concentrated sulphuric acid, the albumen and both cotyledons exhibiting in some seeds a red coloration; in others, the albumen and one cotyledon a green coloration, the other cotyledon a purplish one. Further variations were also shewn by other commercial seeds, but in every case the tints seemed constant for each variety. It was suggested that this might be due to the fact that the "Kombé" seeds of commerce are derived from different species of Strophanthus; to this supposition additional weight was given by Dr. Blondel's Les Strophantus du Commerce, where three distinct varieties of "Kombé" seeds were claimed to have been found on the market, and to be easily capable of identification by their histological characters. Furthermore, through the kindness of Mr. Holmes, I was able to examine the seeds from three different pods, each pod being obtained from a different district in East Africa. The seeds in one of the pods gave for the most part a green coloration with concentrated sulphuric acid (and these were regarded by Mr. Holmes as the official kind), while the seeds from the other two pods gave a red coloration with the same reagent. An attempt was therefore made to ascertain whether the structure would reveal any characters by which the seeds giving these different reactions could be distinguished. At the very outset, however, it was found that the seeds from one and the same pod exhibited differences among themselves as great as, if not greater than, those indicated by Dr. Blondel. In view of this, it was deemed advisable to examine the seeds from one pod as thoroughly as the means available would allow, and for this purpose those seeds in which the green coloration with concentrated sulphuric acid is the most prominent have been first dealt with, and constitute the subject matter of this paper.

The shape of the seeds is a somewhat variable quantity (Fig. 1); the general outline, although frequently oval (Fig. $1A_1$), may be

sometimes almost ovate (Figs. $1D_1$ and $1E_1$), and even slightly obovate (Figs. $1B_1$ and $1C_1$); they are always acuminate. The size of the seeds, likewise, varies considerably, and, although I have only had a limited number of them at my disposal, I have found the length to vary from 12.5 to 17.5 mm., the width from 3.5 to 4.5 mm., and the thickness from 1.2 to 2.3 mm. The thicker seeds are usually straight, although this is by no means always the case (Figs. 1A, 1B, and 1C), while the thinner ones are generally twisted spirally (Figs. 1D and 1E). The base of the seed is terminated by a fine pointed, rounded, or truncated membranous wing (see Fig. 1), most clearly seen in a longitudinal section (w^{s} , Fig. 2). On the ventral surface ¹ of each seed there is a longitudinal ridge extending from the apex to half or over two-thirds of the way down. This ridge, sharp and steep towards the apex, becomes flatter and wider towards the base in varying degrees, and may occupy a strictly median position, as in Fig. 1B1, but is more frequently deviated to one side towards the apex, either to the right, as in Fig $1E_1$, or to the left, as in Figs. $1A_1$ and $1C_1$; a further variation occurs in some seeds, where the ridge follows a somewhat wavy course, the deviation occurring first in one direction and then in the opposite one, as in Fig. 1D₁. At some point on this ridge the scar of the funicle may be seen as a small white dot (f. sc., Figs. 1A1, 1B1, 1C1, 1D1, 1E1), indicated in the figures by a *black* one; its position is very variable, being in some cases quite near the apex (Fig. 1A,), in others nearly at the centre (Figs. $1D_1$ and $1E_1$); more frequently, however, it is found at some point between these positions (Figs. 1B, and 1C1). The dorsal surface is usually flat or slightly convex (Figs. 1A₂, 1B₂, 1D₂), but sometimes conspicuously convex (Fig. 1C₂) or slightly concave (Fig. $1E_2$; the ventral surface is either convex or nearly flat; in the latter case the median ridge stands out boldly (Figs. 1B₁ and 1B₃). The surface is covered with short, closely-appressed silvery hairs, directed towards the apex of the seed and arranged in longitudinal rows. The term "silky" which has been frequently applied to them is, although quite correct technically, somewhat misleading, as they are stiff and rigid, and resemble hog's bristles far more closely than they do silk fibres. This feature is seen with especial clearness if the hairs be examined under a good lens.

The colour of the seeds varies according to the position of the

¹ The term "ventral surface" is here used to denote that side of the seed which faced the placental surface of the follicle; the opposite side then becomes the "dorsal surface."

observer and of the seed with respect to the incident light. If a seed in which the hairs are in their natural position be placed at right angles to the incident light, and be viewed from the base, it will present a silvery appearance, with just a faint suspicion of a yellowish or grey-green tint; if, on the other hand, it be viewed from the apex under the same conditions, it will have lost all, or nearly all, its sheen, and will appear obviously green, greenishfawn, or brownish-green. In intermediate positions intermediate tints are displayed. When the hairs are scraped off, the same tint as that exhibited by the inverted seed, viewed as above, is seen in all positions, a fact which tends to shew that the green colour of the seed is not due principally to the contents of the hairs, as has been generally supposed, but rather to the contents of deeper-seated layers.

When placed in water the seeds, when left to themselves, float on the surface. They can be caused to sink by wetting them with the fingers, and after soaking they swell, and may be easily separated into three distinct portions, viz. :—

(1) The integuments of the seed (Fig. 3a).

*

(2) The albumen, consisting of a longitudinally-grooved, more or less bi- or plano-convex envelope, of cartilaginous consistence, narrowed into a rounded point towards both ends, gradually towards the extremity directed to the base of the seed, abruptly towards the other extremity (Fig. 3b).

(3) The embryo, consisting of two straight plano-convex cotyledons with their flat sides facing each other, and of a well-marked radicle fitting into the abruptly narrowed end of the albumen (Fig. 3c).

Transverse sections cut through different seeds in the regions where they most resemble each other (generally a little below the middle) are shewn in Fig. 5. The variations noted above in the thickness of the seeds and in the curvature of their surfaces are , well shewn, while the central white core of the median ridge, here due to the vessels of the raphe, is also evident. Sections through upper, middle, and lower portions respectively of a well-developed seed are shewn in Fig. 4, and represent fairly well the variations which obtain, broadly, in most seeds. It will be observed, for instance, that near the apex the section is at its flattest, whereas it is relatively thickest near the base. At the periphery of a transverse section the hairy epidermal layer of the seed-coats is visible, and is subtended by a thin green line, the latter dividing at the raphe so as to form an internal, as well as an external, border to it. Immediately under the above, which together constitute the integuments of the seed (s. c., Figs. 2, 4, and 5), the albumen is situated (alb., Figs. 2 and 4). It presents a somewhat translucent appearance in section. I have not found its average thickness to vary much in different specimens (see Fig. 5), although the local variations at different points of the same section are very considerable, as will be shewn later. Surrounded by the albumen, the two plano-convex cotyledons are seen, unless the sections have been cut near the apex of the seed, in which case the radicle alone will be visible. The cotyledons are always more opaque than the albumen, and vary considerably in thickness, being sometimes very much thicker than the latter (Fig. 5a), sometimes nearly as thin (Fig. 5e), but usually considerably thicker (Figs. 5b, 5c, 5d). In addition to these features, a longitudinal section at right angles to the plane of the two cotyledons (Fig. 2) shows the extent of the raphe (ra., Fig. 2), the position of the growing point (g. p., Fig. 2), the membranous wing (w^{g} ., Fig. 2), and the upward direction of the epidermal hairs.

We will now proceed to examine in detail the structure of the integuments, albumen, and embryo.

The integuments of the seed, seen in a transverse section mounted in pure glycerin, shew two distinct regions (Fig. 6), an external one, composed of a single layer of cells and constituting the epidermis of the seed (ep., Fig. 6), and an internal one, composed of several layers of compressed cells, and forming a narrow green band much thinner than the epidermis (gr. l., Fig. 6). On inspection of Fig. 6 it will be seen that these integuments are thrown into numerous sharp folds and furrows, most of which follow similar irregularities in the albumen. In some cases, however, the folds consist only of integuments, the epidermis in such a case forming the external portion of the fold, and the inner layers its internal one (f., Fig. 6). After soaking in water, or better, after mounting in chloral-hydrate solution, the outer cells of the green band just mentioned unfold at intervals, and give rise to an undulating outline (Figs. 7, 8, and 9 on the right). If the epidermis, separated by soaking in water, be examined in surface view. these undulations appear as ridges and furrows (Fig. 10), the latter being filled up by dense aggregations of the upwardly directed epidermal hairs, while the ridges are very much more sparingly clothed.

The structure of the epidermal cells shews a rich variety which seems, for some inexplicable reason, to have been overlooked by previous workers, even by such eminent experts as Professor Hartwich, Professor Louis Planchon, and Dr. Hanausek, although the last came very near to giving the true explanation, which, unfortunately, he afterwards withdrew on the publication of Dr. Nevinny's researches.

In the simplest case, illustrated by the cells of the median ridge near the apex of the seed, we get a cell with its side walls strengthened by a yellow, lignified and striated hoop of thickening (l. hp., Fig. 10'), which appears to be lined on its internal face by a delicate cellulose membrane. The outermost portions of these side walls, as well as the outer and inner walls of the cell itself, consist of cellulose. The outer wall is prolonged, generally at its upper end, into a short hair also consisting of cellulose (h., Fig. 10'). In Fig. 11 a slight increase in complexity is shewn, together with a lengthening of the hair; here the region of lignified thickening is more extensive, and consists, in addition to the lateral hoop just mentioned, of a ring at the bend of the hair (r., Fig. 11), and of an ascending band joining the hoop to the ring (asc. b., Fig. 11), while the internal face of the hair on the side next to the seed-surface is floored by a lignified strip running along its entire length. The larger proportion of the epidermal cells, however, belong to the types shewn in Fig. 12 (perspective side view), Fig. 13 (longitudinal optical section), Figs. 14, 15, 16 (surface views, hairs broken off), and Fig. 17 (perspective surface view). In these the dome-shaped outer cellulose wall of the cell is usually traversed by two or more curved and lignified bands joining at the apex of the dome to form a ring (r., Fig. 17), the latter being situated, as in the previous case, at the point where the cell becomes narrowed into the hair with a sharp bend; from the ring two or more branches arise and ultimately unite to form the more or less uniform. thickened and lignified strip running along the whole length of the internal border of the hair; the point of the hair is also frequently lignified internally. In Fig. 18 a hair is shewn as seen from the outer surface, and in Fig. 19 the fragment of another as seen slightly from the side. In Fig. 20 (optical longitudinal section), and in Fig. 21 (tangential section, perspective), cells are shewn in which bands of thickening occur in other positions also; these seem to be the "rafter-like" (balkenförmige) thickenings which Professor Hartwich observed in glabrous seeds from Lagos and Zambesi, but which he stated to be absent in the hairy varieties of Strophanthus. Such thickenings are certainly comparatively rare, but the ascending bands noted above might be looked upon as "rafter-like" thickenings. Other variations occur, but these are the principal ones.

The thickened hoop of an epidermal cell in longitudinal and transverse section appears as two yellow and striated areas, flat on their external faces where they were in contact with similar faces of adjoining cells, and convex in varying degrees internally (hp. s., Figs. 10', 11, 13, 20). In the tissue itself these areas always appear more or less bi-convex (hp. s., Figs. 22 to 27, ep., Figs. 8 and 9), owing to the juxtaposition of their flat external faces. On the shape and relative height and breadth of these bi-convex areas as seen in tranverse section, and on their distance from each other, great stress has been laid by many pharmacognosists, as a means of distinguishing seeds of different origin, but it will be well to note that the variations which may occur, not only in seeds derived from the same pod, but even in the selfsame seed, are by no means inconsiderable. One of the most interesting of these variations is shewn by the cells situated in the furrows and on the ridges respectively, the side walls in the latter being, as a rule, further apart, and their lignified areas more strongly developed than in the former; the outline of these areas, moreover, is usually more sharply bi-convex in the furrows than on the ridges, where it is more nearly oblong in the middle portion, narrowing down only near the top and bottom; this general rule, as one might expect, does not hold with mathematical accuracy, as Figs. 23 and 24 clearly shew. It is to be noted, further, that when the section happens to pass through one of the ascending bands which is fairly upright, these lateral thickenings will naturally appear much more elongated and parallel-sided (asc. b., Figs. 23, 25, and 13 longitudinal section). In sections which are not too thin one frequently sees the arch formed by two of these ascending bands on their way to the apex of the dome (see Fig. 26, where the inner thickened portion of the hair, l.h.b., is also shewn in section). The lateral walls of the cells situated over, or in close proximity to, the greater part of the median ridge of the seed are close together, and their thickened portions are nearly always elongated and seldom much swollen, shewing a slightly fusiform outline at best (ep., Fig 9 on the left); the ascending bands of thickening must be very numerous here, for a section through this region always shews a large number of the much elongated areas noted above (ep., Fig. 9). In surface view the epidermis is seen to consist either of polygonal axially elongated (Figs. 14 to 17.

21 and 28), or nearly isodiametric (Fig. 29) cells, or of axially elongated ones with tortuous walls (Fig. 30). The yellow striated hoops of the side walls give to these cells the appearance of sclerenchymatous tissue, but careful focussing will reveal the curvature of these walls and also their outermost (here seen uppermost), thin cellulose portions, usually easily recognised by being somewhat wavy (cell w., Figs. 8a¹, 14 to 16, and 21). Figs. 28 to 30 and Fig. 8a² represent ideal tangential sections through the thickest parts of the cells. It will be easily realised from these how very variable the distances between the longitudinal walls are (the section of the latter being, of course, those which are seen in transverse section). Fig. 30 is a very fair average of the cells which occur over the median ridge. The left-hand portion of Fig. 28 represents part of a ridge and the right-hand one of a furrow, the arrow indicates the direction of the slope from one to the other. It will be noticed that not only are the cells in the furrow more narrow than those on the ridge, but they are altogether smaller and consequently more numerous.

The other features of the epidermis visible in surface view, although more difficult of observation, can nevertheless be quite satisfactorily made out by careful focussing. The lignified bands, which run from the thickened hoop on the lateral walls to the apex of the cell, are best seen on the slopes, as they are frequently damaged on the ridges (Fig. 16), while in the furrows the overlying hairs hide them from view; the rings at the apices of the cells are, however, nearly always perfectly apparent. The hairs are also easily made out, their structure has already been described at some length, and the following particulars will be sufficient to complete the description. Their length varies usually from 0.5 to 0.8 millimetres, but they may be very much shorter, as, for instance, towards the apex of the seed, especially on the ventral ridge; these occasionally occur also mingled with the longer hairs on any part of the seed. I have never found any hair exceed a millimetre in length, although Dr. Nevinny found them to be several millimetres long in some (Kombé?) seeds. When a hair is broken off, the separation takes place at the apex of the epidermal cell, at the place where the bend and the above-mentioned lignified ring occur, in such a way that the ring is carried away also, although the latter is not usually found in a hair which has been so broken off (Fig. 19), but it may be seen in Fig. 18 (which is somewhat exceptional in having the perfectly intact ring attached).

*

Every epidermal cell, I think I may say without exception, possesses a hair, and we have seen that these cells are more numerous in the furrows than on the ridges. These two facts will help us to realise how the more or less regular aggregations of hairs in longitudinal rows come about, for not only will the floors of the furrows be more thickly clothed, but the hairs on the slopes of the ridges will also contribute to make of these furrows longitudinal cushions of superposed hairs. These observations have, of course, been made on seeds which had been soaked in water or otherwise swollen, but in the dry seeds the case would only be accentuated, inasmuch as the swelling of the seed-coats simply converts the sharp folds of the latter into gentle undulations.

There is still one point which is perhaps worthy of mention. The statement has been made by Dr. Blondel, and corroborated by Professor Louis Planchon, that the outer walls of the epidermal cells are frequently so shrunken that they touch the inner ones. I have observed this in comparatively rare instances only, and these almost entirely limited to transverse sections. If a transverse section stained with hæmatoxylin be examined, a possible explanation of this appearance is found (Fig. 27); here this apparent dip is certainly due to the hoop seen in optical section, for its light vellow colour stands out boldly from the blue line due to the remains of the outer cellulose portions of the walls. The only reason I can suggest, for this apparent inward dip of the outer margin of a more distant portion of the hoop, is that this portion has been pushed inwards by the razor in cutting the section, or else that the portion nearer to the observer has been pulled outwards.

The remaining layers of the seed-coats consist entirely of thinwalled cells, compressed at right angles (except in some of the folds) to the surface of the seed; when examined in pure glycerin they appear merely as a pigmented band whose component elements cannot be made out; after soaking in water for some time, however, or after treating with chloral-hydrate solution, considerable unfolding of the cell-walls takes place in certain regions, giving rise, in transverse section, to the wavy outline already mentioned and shewn in Fig. 8 and 9 (*Int. int.*). All the cells seem to be very much alike, and to differ mainly in the amount of unfolding which they have undergone after soaking (although the cells immediately under the epidermis are frequently smaller in size); they have very thin cellulose walls and slightly but distinctly thickened corners; when completely unfolded, as they frequently are in the cores of the ridges, they shew a polygonal outline in transverse section (Figs. 8 and 9), and also in a radial longitudinal (Fig. 31), and in a tangential one (Fig. 32); it is very difficult, however, to get satisfactory radial and tangential sections, for the cells are so wedged in between each other that the walls of an increased number of them come to be very nearly in the same plane, giving rise to the appearance shown in Fig. Sa³. The general arrangement of the various layers, in a soaked seed, is somewhat as follows: the innermost layers of cells are not well defined, and appear to be somewhat mucilaginous; between these and the exterior we get a fairly continuous band consisting of several layers of slightly expanded cells which persistently retain their green or brownishgreen pigment; this band grazes the epidermis in the furrows, but its limit is not very well marked under the ridges, for here the outer cells merge gradually into the looser arrangement which obtains in the folds. Intercellular spaces are absent throughout. Under the median ridge the same three regions are seen with especial distinctness, the middle pigmented band (gr. b., Fig. 9)being here sharply marked off from the innermost colourless layers and from the outer well-developed loose tissue (par., Fig. 9); in the latter, the vascular strand of the raphe is situated, if the sections be taken below the insertion of the funicle (v, b), Fig. 9). This vascular strand consists of small spiral vessels running in a more or less longitudinal direction; its border is joined to the surrounding loose tissue by a zone of delicate smallcelled parenchyma (sm. c. p., Fig. 9). With regard to the occurrence of spiral vessels, I can confirm Professor Hartwich's observation, which is that they are entirely confined to the raphe. I have not been able to find any indications whatever of laticiferous tubes in any part of the seed-coats, although I have carefully looked for them; these were found by Dr. Blondel in the closely related "hispidus" seeds, where their presence was emphasized by him in the following words :--

"Il (le second tégument séminal) est formé d'éléments aplatis, fusiformes, à parois très minces, souvent sinueuses, ce qui leur donne, lorsqu'elles sont parallèlement accolées, l'aspect d'un écheveau ondulé ou d'un laticifère tortueux à paroi plissée. Or, precisément, il y a des laticifères dans cette couche, si bien qu'il devient souvent très difficile de les voir, ou plutôt de ne pas prendre pour des laticifères ce qui n'en est pas. Ils existent toutefois très réellement, fait rarement observé, à notre connaissance, dans les téguments séminaux; dans les points ou le tégument primaire, soulevé par un pli, laisse un peu de laxité à la seconde couche, on distingue nettement leurs contours, leur paroi mince, leur contenu brun; ils deviennent surtout évidents, à la face ventrale, au niveau de la crête médiane qui continue le funicule. . . .¹ This observation was confirmed by Professor Smith Ely Jeliffe who found "laticiferous vessels" in this tissue (and apparently in both "Kombé" and "hispidus" seeds).

The albumen will next be considered. A transverse section through a dry seed, mounted in glycerin (Fig. 6), shews its external outline to be exceedingly irregular, owing to the presence of grooves and ridges as in the integuments; after soaking, these irregularities become less pronounced, although they still remain very noticeable (see Figs. 7 and 8, which represent a very good minimum of irregularity). It may be advisable at this point to remind the reader that the surface of the albumen, soaked out as in Fig. 3b, always appears grooved, whereas the integuments appear more or less even, this latter condition being due, as has already been pointed out, to the filling up of the furrows by epidermal hairs.

The outermost layer of the albumen consists of cells which appear rounded-polygonal, gelatinous and fairly thick-walled in surface-view (Figs. $8a^5$ and 34), more or less cubical in radiallongitudinal and transverse sections; from Fig. 8^5 , which was sketched from a section cleared in chloral-hydrate, it will be seen that only the outer walls are uniformly thickened, the lateral ones thinning down like wedges towards the interior. The remaining cells of the albumen, with the exception of those forming the innermost layers, are polygonal, thin-walled, and very slightly thickened, if at all, at the corners (Figs. 8^6 , 35, 36, 37); in tangen-

¹ The following is an attempt to translate this somewhat difficult passage :—" It (the second integument of the seed) is formed of flattened fusiform elements, with very thin walls which are frequently sinuous, this giving them, when they are joined in a parallel manner, the appearance of an undulating skein or of a tortuous laticiferous (tube) with a wrinkled wall. Now, it so happens that there are laticiferous (tubes) in this layer, so that it is often a difficult matter to see them or rather not to mistake other structures for laticiferous (tubes). They are, nevertheless, certainly present, a state of things seldom observed, as far as we are aware, in seed integuments; in the places where the first integument, raised by a fold, allows a little looseness to the second layer, one clearly distinguishes their outlines, their thin walls, their brown contents; they become especially evident, on the ventral surface, at the level of the median ridge which forms a continuation of the funicle. . . ."

tial (Fig. 8a⁶), and in radial-longitudinal sections they present exactly the same features. In no case have I been able to detect unequivocal examples of intercellular spaces. The innermost portion of the albumen consists of several layers of much compressed and somewhat mucilaginous cells, very similar to those forming the internal limit of the integuments. What has just been said seems, at first sight, perfectly simple and straightforward, but, as a matter of fact, these albumen cells have given rise to a refreshing variety of opinion among different workers; for instance, Dr. Hanausek described them as "polygonal, exceedingly delicate-walled and closely fitted together," while, according to Dr. Nevinny, they are "isodiametric or somewhat tangentially elongated, and possess colourless, shining collenchymatous walls" (Fig. 38). Dr. Blondel, on the other hand, succeeded in finding no less than three different kinds of cells in three corresponding varieties of Kombé seeds; in the first variety these were stated to be "rounded with extremely thin walls" (Fig. 39), in the second, "rounded with tolerably thin walls" (Fig. 40), and in the third, "rounded and moderately (médiocrement) thick" (Fig. 41). Professor Hartwich, in his first paper, found them to be "fairly large and thin-walled," while in his second one, the cell-walls of the embryo, in Strophanthus seeds generally, were stated to be "thin when compared with those of the endosperm" (see Fig. 42). Dr. Fraser published no description of the structure of the seeds, but he gave figures in which the walls of the albumen cells were depicted uniformly thin in some cases (thn., Fig. 48), in others uniformly thick (thk., Fig. 43), in others still, irregularly thickened (irr., Fig. 43). Finally, Professor S. E. Jeliffe figures them as "polyhedronal" and fairly thin-walled (about as in Fig. 33), and also mentions the fact that two of Blondel's three types were observed by him. Now, it is possible to get a good deal of variation in the same section according to the conditions of observation. An account of various observations made under different conditions, and with different reagents, will accordingly be given.

In a moderately thin section, cleared with chloral-hydrate, the cell-walls appear thin, as has already been stated, but in a somewhat thicker section some of the walls appear thick, especially if the contents have not been thoroughly cleared; if a section which has been thus treated be deeply stained with hæmatoxylin, we get a suggestion as to the reason for this thick-walled appearance (see Figs. 35, 36, 37). It is here seen that the walls are more or less wavy (*ps.-t.*, Figs. 37), or slanted, and by careful focussing the

* *

section of the actual wall, which might at first be taken for a middle lamella, is seen to swing from one side to the other; where some of the cell-contents have been preserved, the appearance is exceedingly deceptive (see Fig. 37). Seeds which have been soaked in water give similar results. When the transverse section of a dry seed is examined in pure glycerin most of the cells are seen to possess considerably creased walls (Fig. 44), and to present in some places a uniformly thick-walled and gelatinous appearance, in others the walls appear quite thin, and in others still, they appear to be irregularly thickened-this agrees quite well with Dr. Fraser's figure. If the section be now treated with chloral-hydrate, it will be noticed that the walls stretch out, so that the creases come to be represented by the undulations noted above, these curvatures being retained more especially by the walls lying at right angles to the plane of the section. With water, the same features can be observed, but only after some time, as the action is slow; it is necessary, moreover, to get a very thin section, as otherwise the disintegration of the cell-contents will obscure the result. I have not been able to observe any considerable swelling of the cell-walls in the last two cases, but it is, nevertheless, probable that the cellulose of these walls has undergone some modification, for they appear more gelatinous and highly refracting than ordinary cellulose, and are stained red by a solution of Ruthenium red in 10 per cent. aqueous lead acetate; 1 the last-named reagent, moreover, seems to make the cell-walls more opaque, and the cells of a section, mounted directly in this medium, accordingly present the thick-walled appearance at its best. In Fig. 45 the action of potash is shewn. In all these cases, except the last, it should be specially emphasized that the cell-walls do not appear thick if the section be thin enough.

These are the general features of the larger number of the albumen cells; the modifications exhibited by the cells of the outer and inner layers have already been briefly mentioned and need no longer detain us, but a few additional minor details may not be inappropriate. The cells, in the sharply curved portion of the albumen at the lateral edges of the seed, are very

¹ This reagent, which was very kindly brought to my notice by Prof. Greenish, has been recommended for the detection of mucilage, and although it probably stains other substances, it has done me good service, inasmuch as it affects all the unlignified walls of the seed in the same way as above, whether they belong to the epidermis or to the inner layers of the seed-coats, to the albumen, or to the embryo, whereas it leaves most of the cell-contents unstained.

often tangentially elongated (*i.e.* flattened at right angles to the surface of the seed), and sometimes slightly thickened (see Fig. 33, which was sketched from a cleared section). The cells of the albumen, situated under the raphe, are seldom much creased, and appear thin-walled under any conditions.

As in the seed coats, I have found laticiferous tubes entirely absent, although the puckering of the gelatinous outer surface may form in certain places gutters which, at first sight, look like thick-walled and striated tubes. A very remarkable case in point is shewn in Figs. 34 and 46 wr.; this was found on the outer surface of the albumen, just over the tip of the radicle, the section being a somewhat oblique longitudinal one, which had just grazed the surface in question. Fig. 47 is an end view of the outer surface of that part of the albumen which covers the rootcap; from an inspection of this figure it will be seen how a case such as that shewn in Figs. 34 and 46 might arise.

In describing the embryo the cotyledons will be first dealt with, then the radicle.

The cotyledons have, as already stated, a planoconvex outline in transverse section. At about the central point of each one of them a more or less semilunar series of procambial strands occurs (*proc.*, Fig. 6), while lateral branches, cut through at varying angles, are seen making their way to the margins of the cotyledon (*latl. b.*, Fig. 6). Around the central procambial series delicate laticiferous tubes are present (*lat. t.*, Fig 49), which are most abundant on the outer convex side; not infrequently they may be also seen accompanying the lateral branches or making their way to the external, or, more rarely, to the internal border of the cotyledon. Between the two cotyledons there is frequently a pad of mucilage (*mu.*, Fig. 6).

The outer epidermis of each cotyledon consists of small thinwalled cells which are cubical when viewed in transverse (*out. ep.*, Fig. 49) and longitudinal sections (*out. ep.*, Fig. 50), polygonal in surface view (Fig. 8a⁸). The inner epidermis is similar (*in. ep.*, Fig. 49), but radial elongation (*i.e.* elongation at right angles to the plane of the cotyledon) frequently occurs here. In both cases the external walls have a tendency to be mucilaginous and slightly thicker. The cells of the ground tissue are polygonal in outline and extremely thin-walled (see Figs. 49, 50, 54 to 56); distinct intercellular spaces are present. The direction of greatest elongation in these cells varies somewhat, but it may be stated broadly, that they are axially elongated in the midrib (*par.*, Fig. 50), and radially elongated elsewhere, especially in the inner half of the cotyledon.

The elements in the area enclosed by the circle-segment, of which the procambial strands form the arc, are axially elongated and of small cross-section; the procambial strands themselves are evident as groups of exceedingly small-celled prosenchymatous elements (*proc.*, Fig. 49).

The laticiferous elements ¹ consist of somewhat sinuous unsegmented tubes, which branch but do not anastomose (*lat. t.*, Figs. 49 and 50); around the growing point of the stem they form a dense felt (*lat. w.*, Fig. 48), which, in transverse section, appears exceedingly like a web of mycelial hyphae; their walls have a gelatinous and somewhat swollen appearance when mounted in glycerin (Fig. 52) or chloral-hydrate (Fig. 51). Ruthenium red in lead acetate solution colours them bright red. All attempts to isolate these structures have proved abortive; I have only obtained small continuous fragments at best.

The radicle is terminated by a well-marked root-cap (*r.c.*, Fig. 48). Its ground parenchyma consists of exceedingly thin-walled isodiametric cells, smaller than those of the cotyledons. Distinct intercellular spaces are present. The procambial strands consist of prosenchymatous elements, similar to those found in the cotyledons; these strands form a hollow cylinder, which appears as a ring in transverse section and as two parallel bands in longitudinal section (*proc.*, Fig. 48). Around the external margin of this hollow cylinder numerous laticiferous tubes occur; these run longitudinally for the most part, although a branch may be occasionally seen making its way to the exterior; branching, however, is much less common here than in the cotyledons.

The cell contents.—These are best defined and most easily made out in the cells of the embryo, which will, accordingly, be taken first.

A cell of the general parenchyma of a cotyledon, sketched from

¹ The laticiferous tubes of the natural order to which Strophanthus belongs (Apocynaceæ) have been shewn to be multinucleate structures, and therefore not true "elements." In modern English text-books these tubes are known as "cœnocytes," Van Tieghem calls them "articles." Before their multinucleate condition was made known they were looked upon as true elements or laticiferous "cells," and this is the term used by de Bary in his Comparative Anatomy of the Phanerogams and Ferns, pp. 190–199. To this work the reader is referred for a complete and remarkably accurate account of laticiferous tubes and their development. It is hardly necessary to add that I have not attempted to establish the "cœnocytic" character of these tubes in the seeds under examination.

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a section mounted in pure glycerin, is shewn in Fig. 56; if such a section be examined as soon as it is mounted, the contents are seen to consist of rounded-polygonal, highly refractive bodies (al., Fig. 56), embedded in a hyaline ground mass (g.m., Fig. 56); small starch grains are also present in variable quantity, but oil drops are invisible as such. If the section be treated with ether before mounting in the above medium, no very noticeable change is observable, and I am totally at a loss to understand how the large vacuoles figured in Plate VI., Fig. 8, of Dr. Fraser's monograph can have come about; in water, disintegration of the cell-contents takes place, and the whole field becomes crowded with innumerable oil drops. It will be advantageous, at this stage, to examine each of these items separately, and in some detail; they will be dealt with in the following order :—

1. Ground-mass, including oil.

2. Aleurone grains (the rounded-polygonal refractive bodies noted above).

3. Starch.

1. The ground-mass consists of the oily protoplasmic network in which the solid bodies are embedded; somewhere in its substance the nucleus (n., Fig. 55) is found. The oil occurs in very intimate connection with the protoplasm, and perhaps in actual chemical combination with it, for with the highest powers at my disposal I have been unable, in a glycerin mount, to detect the slightest indication of oil drops; water seems to destroy this combination, hence the appearance of oil drops with aqueous media. If a section, deprived of its oil by means of alcohol and ether, be warmed with chloral-hydrate solution, and subsequently stained with hæmatoxylin, it is occasionally possible to get a cell in which the protoplasmic remains are apparent as an exceedingly delicate network (Fig. 55). A better reagent still than chloral-hydrate is concentrated aqueous sodium phosphate, which dissolves the aleurone grains completely, but not the protoplasmic network, nor the starch. In thin sections the localisation of the oil can be made out with tincture of alkanet, or even with a 1 per cent. aqueous solution of osmic acid; for although in the latter case oil drops separate out and obscure the reaction, yet the unstained aleurone grains in situ stand out fairly well.

2. The aleurone grains, examined at once in slightly diluted glycerin, appear as homogeneous, rounded-polyhedral, highly refracting bodies (Fig. 57); but if left in this medium for a short time, solution gradually takes place from the outside to the interior and only minute rounded bodies (globoids) enclosed in a membrane are left behind, as pointed out by Prof. Hartwich (see Fig. 59). A similar result is arrived at with very dilute potash, but in this case the effect is instantaneous and more sweeping, for as soon as the reagent reaches the grain its contents swell and the membrane bursts, liberating the contained globoids. Alcoholic iodine stains the aleurone grains brown; iodine water does also, but in irregular patches (Fig. 58); Millon's reagent colours them red: their size varies from 2μ (0·002 mm.), or less, to 15μ (0·015 mm.), but the usual range is from 7.5μ (0·0075 mm.) to 10μ (0·01 mm.); in the larger grains the globoids are numerous, whereas in the small ones they may be solitary (Fig. 59); crystalloids are absent throughout. All observations with aqueous reagents should be made on sections which have had their oil removed, as the latter forms a great hindrance to the study of the aleurone grains.

3. The starch grains may occur in considerable abundance in some cells, notably in those of the parenchyma of the midrib; they are always very small, but can be made quite evident with a solution of iodine in chloral-hydrate (Fig. 54).

The contents of the laticiferous tubes are finely granular, and, together with those of the procambial strands, are stained deep red by Ruthenium red in lead acetate; this behaviour is exceedingly convenient, as it enables one to determine the distribution of these structures. Another fact which this reagent makes evident is, that along the line joining the ends of the procambial arch there is a series of elements with darkly staining contents; these elements are possibly the rudiments of an internal bast.

We now come to the most difficult part of our task, viz., the examination of the albumen cell-contents.

From Figs. 44 and 53 it will be seen that the cell-contents are chiefly limited to a layer lining the walls of the cells, although it is quite easy to find cells in which bridles are seen traversing the central vacuole (*br.*, Figs. 37 and 53); this central space appears to be quite empty, and I have repeatedly tried oil and mucilage reagents without avail. The protoplasmic ground-mass has a peculiarly stringy and gelatinous appearance, and is, in the dry seed, closely attached to the wrinkled cell-wall (Fig. 44). It resists the action of chloral-hydrate to a considerable extent, and, when this reagent is applied as directed above in the case of the embryo, subsequent staining brings out the details shewn in Fig. 37. Under these conditions it no longer remains in contact with the cell-walls, the latter having expanded more than the groundmass itself; the oil is intimately bound up with it as in the embryo, and no oil drops are visible in a glycerin mount. Embedded in this gelatinous substance, numerous starch grains occur (st., Fig. 53); the size of these may vary from 10μ (0.01 mm.) to minute specks too small for measurement; the latter are generally grouped together round the nucleus (lit. st., Fig. 53); the larger ones are distributed throughout the whole protoplasmic layer, and may be ovoid, nearly triangular, kidney-shaped, or club-shaped (Fig. 60). In the centre of each grain there is usually a depression, which becomes quite evident when the grains are treated with iodine in chloral-hydrate (Fig. 61). The distribution of starch in the albumen is somewhat as follows: The greatest development occurs at the base of the seed, the least at the apex and under the raphe; it is usually more abundant at the lateral edges than on the flat surfaces. In all these places it is most abundant in the middle portion of the albumen, diminishing appreciably towards the exterior, and very considerably towards the interior.

Besides starch, proteid bodies,¹ which look very much like aleurone grains, occur embedded in the protoplasmic ground-mass; these are shewn in Fig. 62. Some of them appear to contain crystalloids as well as globoids. They are usually more variable in size than those of the embryo, and may attain 13.75μ (0.01375 mm.), but seldom exceed 10μ (0.01 mm.). They are best seen after warming a section, deprived of its oil, with Millon's reagent. In any case the existence of aleurone grains in such highly vacuolated cells is suspicious, to say the least, and I cannot help thinking that there is something here which still requires explanation.

The contents of the seed-coats are principally mucilage, and green or brownish-green pigment. Both of these substances are contained almost entirely in the sub-epidermal layers; the mucilage in the cells that expand on soaking, and also in the innermost layers; the pigment, which probably consists of chlorophyll, in all the cells to some extent, but especially in the region which has

¹ Everyone who has published a description of the structure of Strophanthus seeds admits that they contain proteids or aleurone grains in the albumen, but there the agreement ends. Prof. Hartwich says in his second paper that he has found the aleurone grains of Strophanthus agree point for point with Herr Lüdtke's leguminous type. Now, I have read through Lüdtke's leguminous types in *Pringsheim's Jahrb.*, and also in the *Berichte der Pharmaceut. Gesellschaft*, and the only reference I can find to albumen (endosperm) cells is to the "*Aleuronschicht*" or outermost layer only of the albumen (endosperm) where the aleurone grains are very small, and have no inclusions. been previously indicated. The contents of the epidermal cells and of the attached hairs are insignificant. I have, indeed, occasionally observed a few chlorophyll grains in some cells, but these are very scanty at best, and most frequently they do not occur at all.

Calcium oxalate crystals are absent from every part of the seeds under examination.

The Sulphuric Acid Reaction.—The sections when treated with concentrated sulphuric acid invariably exhibit an unequivocal green coloration in the albumen, but the behaviour of the cotyledons is far from constant. In many cases both cotyledons, after a minute or two, become some shade of green, but it is very seldom that they are both tinted exactly alike. In some seeds, on the other hand, the cotyledons, under the same conditions, exhibit red mottlings distributed through the green mass, and in others one cotyledon became olive-green while the other cotyledon exhibited a magenta-red colour. These irregularities had been pointed out by Profs. Louis Planchon and Schlagdenhauffen,¹ and their observations appear to be as accurate as they are remarkable. It is evident from this that there is some close connection between the green reaction and the red one, and also that seeds shewing different colour reactions in the cotyledons alone, need not necessarily belong to different species. The epidermal cells of the seed-coats become dark brown in this reagent, and the lignified portions of their walls enormously distended; the brown colour is probably due to the charring of the lignified portions.

In the albumen the green-staining portion appears to be the protoplasmic ground-mass, but, owing to the swelling and distortion of the tissues, precise determination of this fact is not possible. In the embryo an expression of opinion, based on observations of the sulphuric acid reaction, resolves itself into little more than mere guess-work; still, it is not unreasonable to suppose, as Dr. Nevinny has done, that here, as in the albumen, the reacting principle is contained in the protoplasm. This supposition is confirmed by the fact that the oil, the aleurone, and the starch, when dealt with separately, are not affected in this way.

¹ See Sur un Strophanthus du Congo français. Par MM. les Professeurs Schlagdenhauffen et Louis Planchon, 1897, where an account of all the work that has been done on the colour-reactions of a large number of seeds will be found.

SUMMARY AND CONCLUSION.

An examination of typical East African "Kombé" seeds, all obtained from the same pod, reveals the following facts :---

The seeds vary considerably in size and shape. A ventral, more or less median ridge, extends from the apex of each seed to half or over two-thirds of the way down; somewhere on this ridge the funicle-scar is found, but its position is variable. The hairs on their surfaces are stiff and silvery, point upwards, and are arranged in longitudinal rows. The colour of a scraped seed is some shade of green or brown-green, that of the intact seeds varies with the position of the observer with regard to the seed and to the incident light, this being due to the disposition of the hairs. By soaking, the seeds can be separated into three distinct portions -seed-coats, albumen, embryo-whose details can be observed. The seed-coats and the albumen are longitudinally ridged and grooved; the grooves in the seed-coats are filled up by the upwardly directed epidermal hairs. The cells of the epidermis of the seed-coats shew, on careful examination, considerable variations, and, in most cases, a more complicated structure than has hitherto been supposed; their hairs never exceed one millimetre in length; the appearance presented by their side walls in transverse section, although doubtless of diagnostic value, is far from uniform, and should be taken into consideration in comparing different varieties of Strophanthus seeds. The sub-epidermal layers of the seed-coats, in a soaked seed, may be roughly divided into three regions-a thin inner mucilaginous strip, a middle pigmented band, irregularly arranged loose outer aggregations occurring only under the ridges. The cells of all these layers have very thin walls and thickened corners; intercellular spaces are absent. Under the ventral median ridge of the seed these three regions are well marked, and in the outer loose tissue, below the insertion of the funicle, the spiral vessels of the raphe are situated. Spiral vessels occur nowhere else in the seed-coats, and I have found no evidences of laticiferous tissue. The cells of the albumen present very different appearances according to the conditions of observation, but it is very probable that they are polygonal and thinwalled, with the exception of those of the outermost layer, whose outer walls are thickened, and of those constituting the innermost compressed layers. The embryo consists of two straight planoconvex cotyledons, joined by a well-marked radicle directed towards the apex of the seed ; laticiferous tubes occur whose distribution is conveniently made out with Ruthenium red in lead acetate. The cells of the embryo contain aleurone grains and oil in abundance, the latter not visible as such till the section be treated with aqueous reagents; starch also occurs in very small grains, especially in the midribs of the cotyledons. The contents of the albumen are similar to those of the embryo, but more scanty and, with the exception of the starch, difficult to make out; large vacuoles are present; the starch grains may attain 0.01 mm. The pigment of the seed-coats is probably chlorophyll. The action of concentrated sulphuric acid is constant in the case of the albumen, but variable in that of the embryo; the former always exhibiting a green colour in this reagent, the latter varying shades of green, green mottled with red, or green in one cotyledon and red in the other. The taste of the seeds is intensely bitter.

From what has been said it will be seen that these results are most disappointing, inasmuch as every histological character upon which the identification of the different varieties of "Kombé" seeds has hitherto been based, is found, almost without exception, to exist in seeds obtained from one and the same pod; and although I approached the question with every prejudice in favour of Dr. Blondel's conclusions, I have unwillingly been compelled to abandon them one after the other.

In conclusion, I desire to thank Dr. F. B. Power and Professor H. G. Greenish for the advice and assistance they have given me upon the many matters about which I consulted them. To our esteemed president, Mr. E. M. Holmes, F.L.S., I am especially indebted for invaluable help and for kind suggestions during the progress of this investigation.

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EXPLANATION OF FIGURES.¹

FIG. 1. Seeds from the same pod. A_1 to E_1 , ventral surfaces; (*Plate I.*) A_2 to E_2 , dorsal surfaces; A_3 to E_3 , side views; *f.sc.*, scar of funicle. Natural size.

FIG. 2. Longitudinal section at right angles to the plane of the (Plate I.) two cotyledons; s.c., seed-coats; wg., wing; ra., raphe; alb., albumen; cot., cotyledons; g.p., growing point of stem; rad., radicle; st. +, region of albumen containing abundance of starch; st. -, region of albumen containing little starch. × 3 diameters.

- FIG. 3. a, seed-coats (in an inverted position); b, albumen; c, (Plate I.) embryo, separated out by soaking; r.s., extremity of albumen in which the radicle was situated; cot. cotyledons; rad., radicle. Natural size.
- FIG. 4. Transverse sections through upper, middle, and lower (*Plate I.*) portions respectively, of a well-developed seed, Lettering as in Fig. 2. \times 3 diameters.

FIG. 5. a, b, c, d, e. Transverse sections through seeds from the (*Plate I.*) same pod. \times 3 diameters.

FIG. 6. Transverse section, approximately through the middle of a

(Plate I.) seed, mounted in pure glycerin. ep., epidermis of seed-coats; gr.l., compressed pigmented sub-epidermal layers of seed-coats (shewn as a black line); f., folds of seed-coats; ra., vessels of raphe; proc., central series of procambial strands (bundle rudiments); latl. b., rudiments of lateral bundles; mu., mucilage. Other lettering as in Figs. 2 and 4. Epidermal hairs entirely omitted. Diagrammatic. × 20 diameters.

FIG. 7. Transverse section through seed-coats and albumen, from (*Plate I.*) left-hand top-corner of Fig. 6, after treatment with chloral-hydrate solution. Lettering as in Fig. 6. Diagrammatic. × 20 diameters.

¹ The plates illustrating this paper were kindly supplied by Messrs. Burroughs, Wellcome & Co. FIG. 8. Transverse section through the middle of a seed, on the (*Plate II.*) side opposite to the raphe, showing seed-coats,

albumen, and the outer part of one cotyledon. For explanation of numbers see text. × 200 diameters.

FIG. 8a. The numbered portions of Fig. 8, seen in surface view, (*Plate III.*) or in tangential section. \times 200 diameters.

- FIG. 9. Transverse section through raphe and seed-coats on the (*Plate II.*) ventral surface of a seed. par., outer sub-epidermal tissue of seed-coats, consisting of loose parenchyma; sm.c.par., small-celled parenchyma encircling the vessels of the raphe; v.b., vessels of the raphe; gr.b., pigmented layers of seed-coats; h-cush., cushions formed by the hairs in the furrows. Other lettering as in Fig. 8. \times 200 diameters.
- FIG. 10. Surface view of the epidermis of the seed-coats soaked (*Plate III.*) out in water. Somewhat diagrammatic. × 20 diameters.

FIGS. 10', 11, and 13. Optical longitudinal sections of epidermal (*Plate III.*) cells of the seed-coats. *h.*, hair; *l.hp.*, lignified hoop on the lateral walls; *hp.s.*, hoop section; *r.*, lignified ring; *l.h.b.*, lignified portion of hair. × 200 diameters.

FIG. 12. An epidermal cell with its attached hair, seen in perspec-(*Plate IV.*) tive. Lettering as before. \times 200 diameters.

FIGS. 14 to 16. Surface views of same. Hairs broken off. Cell.w., (Plate IV.) outer cellulose portion of lateral walls. Other letter-

ing as before. The cross in Fig. 16 indicates the places where some of the ascending bands have been broken off. × 200 diameters.

FIG. 17. Perspective surface view of same. Lettering as before. (*Plate IV.*) \times 200 diameters.

FIGS. 18 and 19. Hairs of same. br., branches joining lignified (*Plate IV.*) ring to lignified portion of hair. Other lettering as before. \times 200 diameters.

FIG. 20. Optical longitudinal section of an epidermal cell, with (*Plate IV.*) portion of its attached hair. *r.t.*, "rafter-like"

thickening. Other lettering as before. × 200 diameters.

FIG. 21. Optical tangential section of same. Lettering as before, (*Plate IV.*) The cross indicates the places where ascending bands have been cut through. × 200 diameters. FIG. 22. Transverse section through portions of three epidermal (*Plate V.*) cells. *Cell.mem.*, cellulose membrane; *m.l.*, middle

lamella. Other lettering as before. \times 400 diameters.

FIGS. 23 to 26. Transverse sections through portions of the (Plate V.) epidermis of the seed-coats. Lettering as before. \times 200 diameters.

FIG. 27. Optical transverse section of same. For explanation, (Plate V.) see text. \times 400 diameters.

FIGS. 28 to 30. Tangential sections of same. \times 200 diameters. (*Plate V.*)

FIG. 31. Radial longitudinal section through cells from layer 3 (*Plate V.*) in Fig. 8. \times 200 diameters.

FIG. 32. Tangential section through same. \times 200 diameters. (*Plate V.*)

FIG. 33. Transverse section through the albumen at the lateral (*Plate V.*) edges of a seed. Numbering as in Fig. 8. \times 200 diameters.

FIG. 34. Surface view of the external face of the albumen in the (*Plate VI.*) region of the root-cap. wr., gutter-like wrinkle. \times 200 diameters.

FIGS. 35 to 37. Cells of albumen, cleared with chloral-hydrate (*Plates V. & and stained with hæmatoxylin. ps.-t.*, fold of cell-

VI.) wall; br., protoplasmic bridles; vac., vacuoles; n., nuclei; sp., spaces due to removal of cell-contents. \times 300 diameters.

FIG. 38. Cells from the perisperm (albumen). After Dr. Nevinny. (*Plate VI.*)

- FIGS. 39 to 41. Cells of the endosperm (albumen), from three different
- (Plate VI.) commercial varieties of Kombé seed. After Dr. Blondel.

FIG. 42. Endosperm (albumen) of a starch-bearing variety.

(*Plate VI.*) The section is defatted, and the aleurone grains have been removed with water. After Professor Hartwich.

FIG. 43. Cells from the albumen. Taken from Fig. 6, Plate VI. (*Plate VI.*) of Professor Fraser's monograph. × 150.

FIG. 44. Cells from the albumen, mounted in pure glycerin.

(*Plate VI.*) gel.g.m., gelatinous protoplasmic ground mass ; vac., vacuole. × 300 diameters.

FIG. 45. Cell from the albumen, treated with aqueous potash. (*Plate VI.*) \times 300 diameters.

FIG. 46. Longitudinal, somewhat oblique section through radicle (*Plate VI.*) and surrounding albumen. Lettering as before.

Diagrammatic. × 30 diameters.

- FIG. 47. Surface of albumen, from over the root-cap. Diagram-(*Plate VI.*) matic. × 40 diameters.
- FIG. 48. Longitudinal section, through radicle and through base
 - (Plate VI.) of cotyledons, at right angles to the plane of the latter. r.c., root-cap; lat.wb., felt of laticiferous tissue. Other lettering as before. Diagrammatic × 30 diameters.

FIG. 49. Transverse section through the midrib of a cotyledon,

(Plate VII.) out.ep., outer epidermis of cotyledon; par., general parenchyma of midrib; lat.t., laticiferous tubes; proc., procambial stands; in.ep., inner epidermis of cotyledon. × 200 diameters.

FIG. 50. Longitudinal section of outer half of same, at right (*Plate VIII.*) angles to the plane of the cotyledon. Lettering as

in Fig. 49. The cross indicates the point where the branching of a tube occurs. \times 200 diameters.

FIG. 51. Laticiferous tube in transverse section. Mounted in (*Plate VII.*) chloral-hydrate. × 400 diameters.

FIG. 52. The same, mounted in glycerin. \times 400 diameters. (*Plate VII.*)

FIG. 53. Cell of albumen, from which the oil has been removed, (*Plate VII.*) stained with iodine water. st., starch grains; lit.st., small starch grains; al., aleurone grains (?). Other lettering as before. × 300 diameters.

FIG. 54. Cell from the general parenchyma of the midrib of a (*Plate VIII.*) cotyledon, treated with chloral-hydrate-iodine solution. × 300 diameters.

FIG. 55. Cell from the general parenchyma of a cotyledon, cleared (*Plate VIII.*) with chloral-hydrate solution and stained with hæmatoxylin. n., nucleus; prot., network of protoplasmic remains. × 300 diameters.

FIG. 56. The same, mounted in pure glycerin. al., aleurone (*Plate VIII.*) grains; g.m., the oily protoplasmic ground mass. \times 300 diameters.

FIG. 57. Aleurone grains from cotyledons, mounted in pure (*Plate VIII.*) glycerin. \times 500 diameters.

FIG. 58. The same in iodine water. \times 500 diameters. (*Plate VIII.*)

- FIG. 59. The same with extremely dilute potash. *mem.*, mem-(Plate VIII) brane; *gl.*, globoids. \times 500 diameters.
- FIG. 59a. Globoids of same. × 500 diameters.

(Plate VIII.)

- FIG. 60. Starch grains from albumen, in iodine water. \times 500 (*Plate VIII.*) diameters.
- FIG. 61. The same, treated with chloral-hydrate-iodine solution. (*Plate VIII.*) \times 500 diameters.
- FIG. 62. Aleurone grains (?), from albumen, in iodine water. St., (Plate VIII.) adhering starch grains; gl., globoids (?); cr.,

crystalloids (?). \times 500 diameters.

FIG. 62a. Globoids (?) of same. × 500 diameters. (*Plate VIII*.)

THE WELLCOME RESEARCH LABORATORIES.

Plate I.

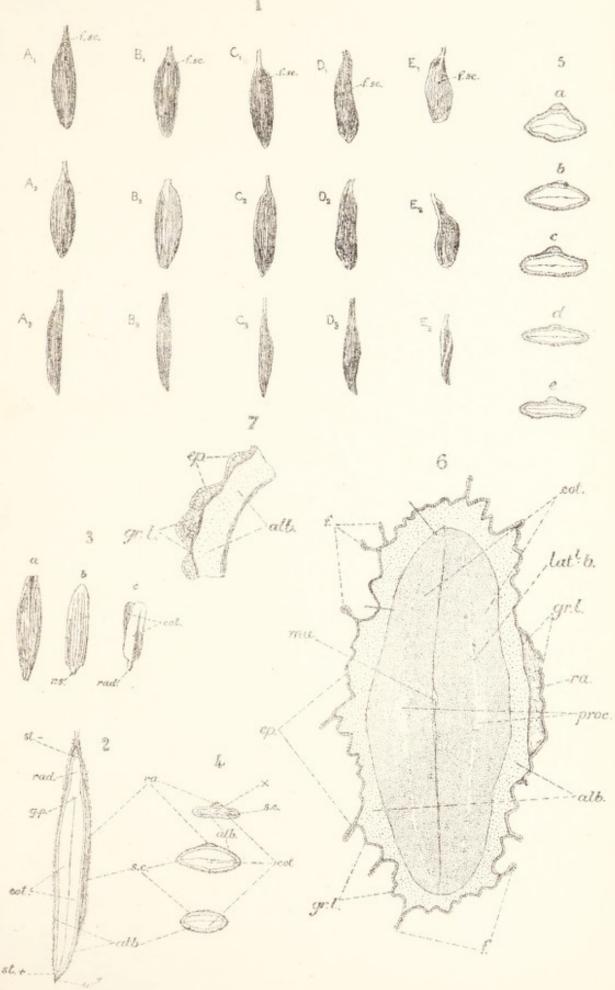
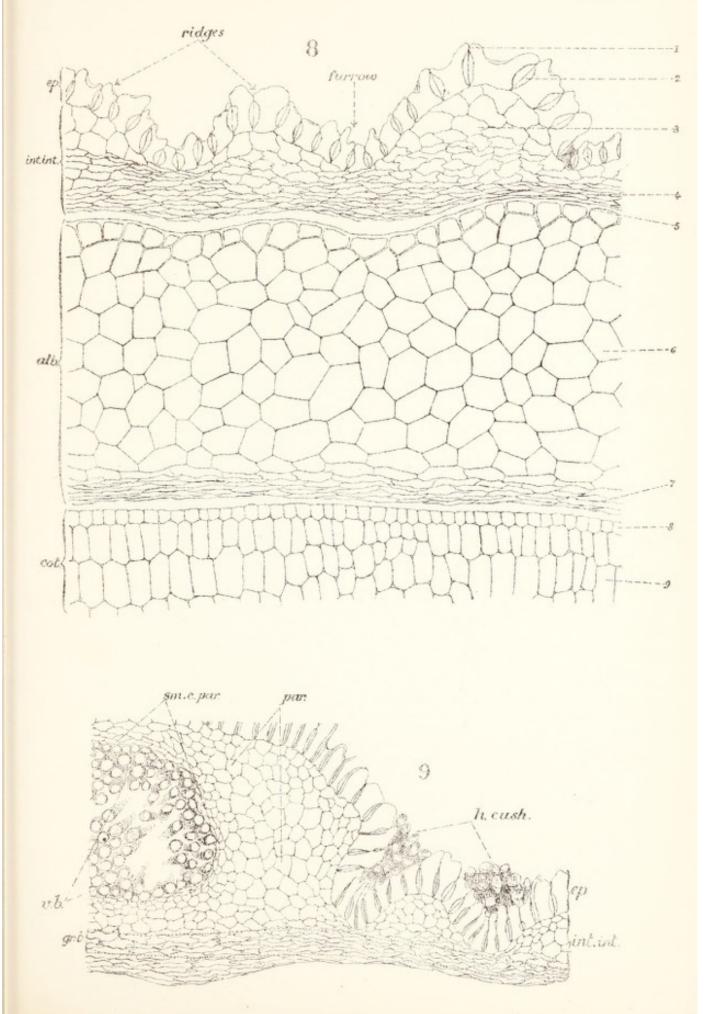
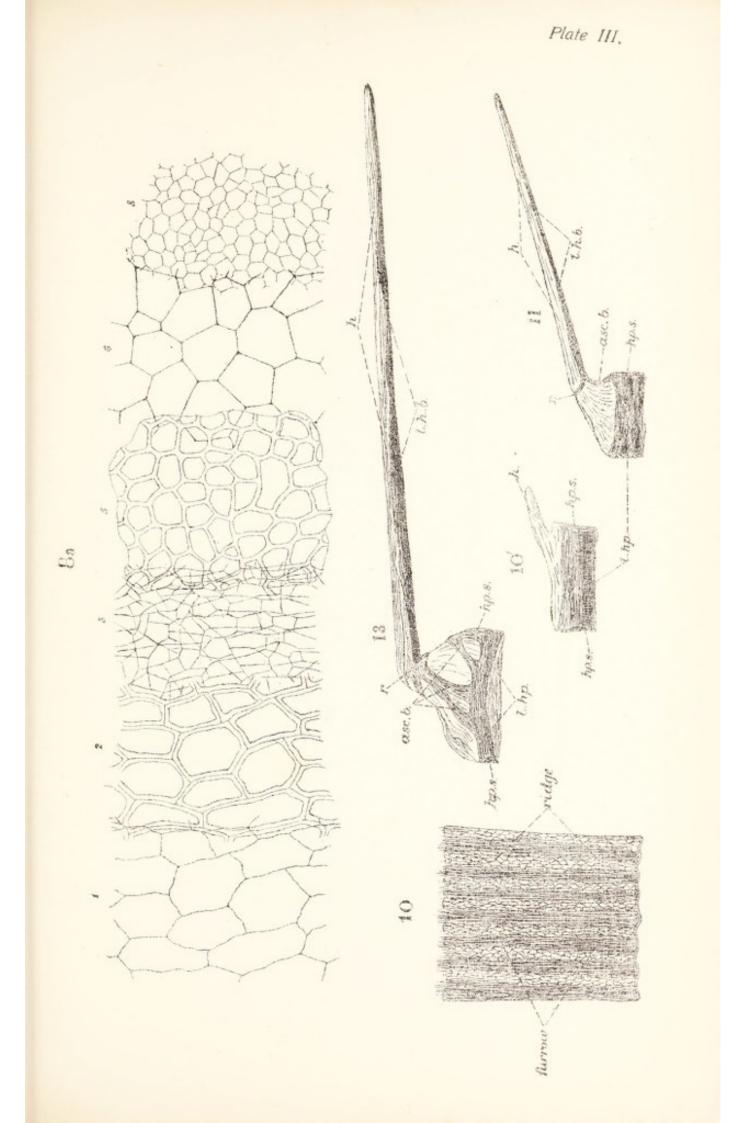




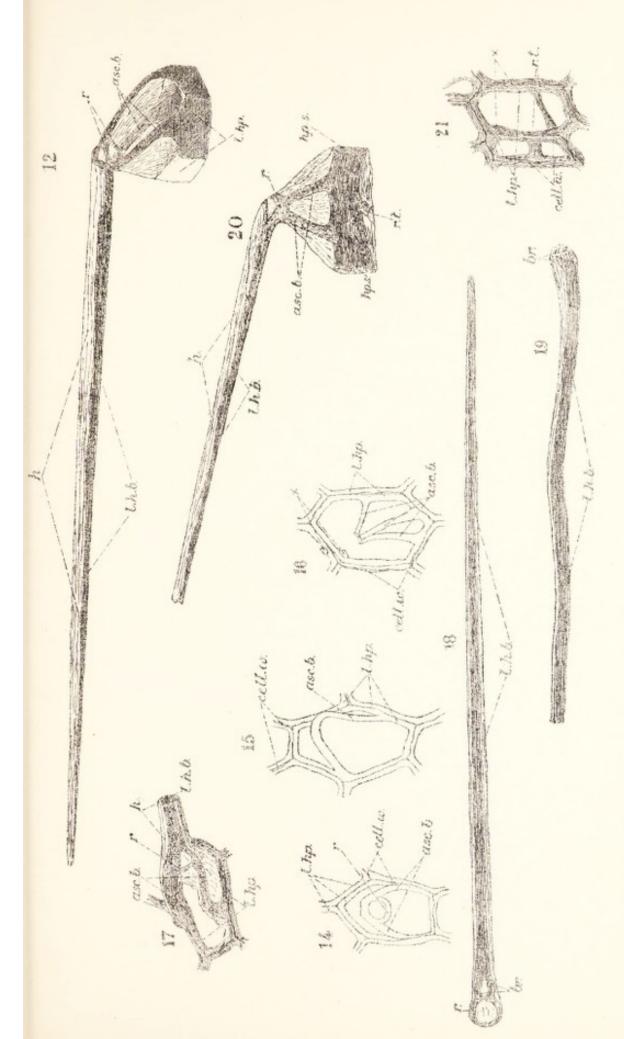
Plate 11.











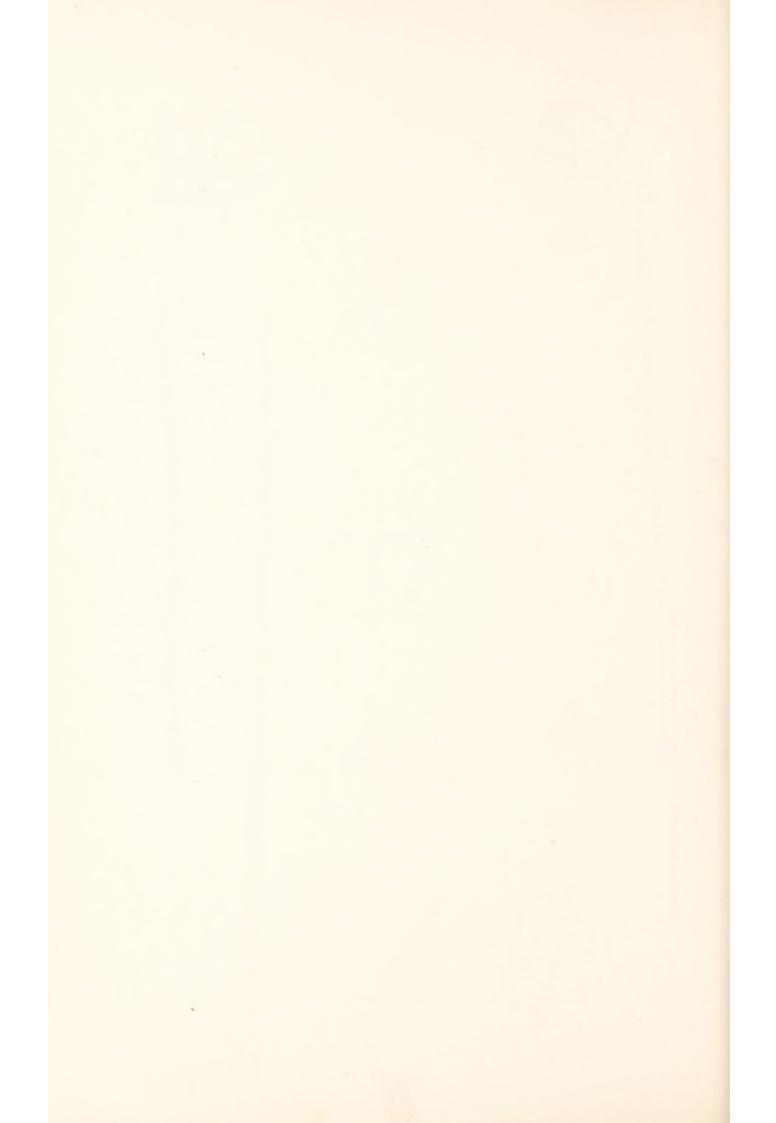


Plate V.

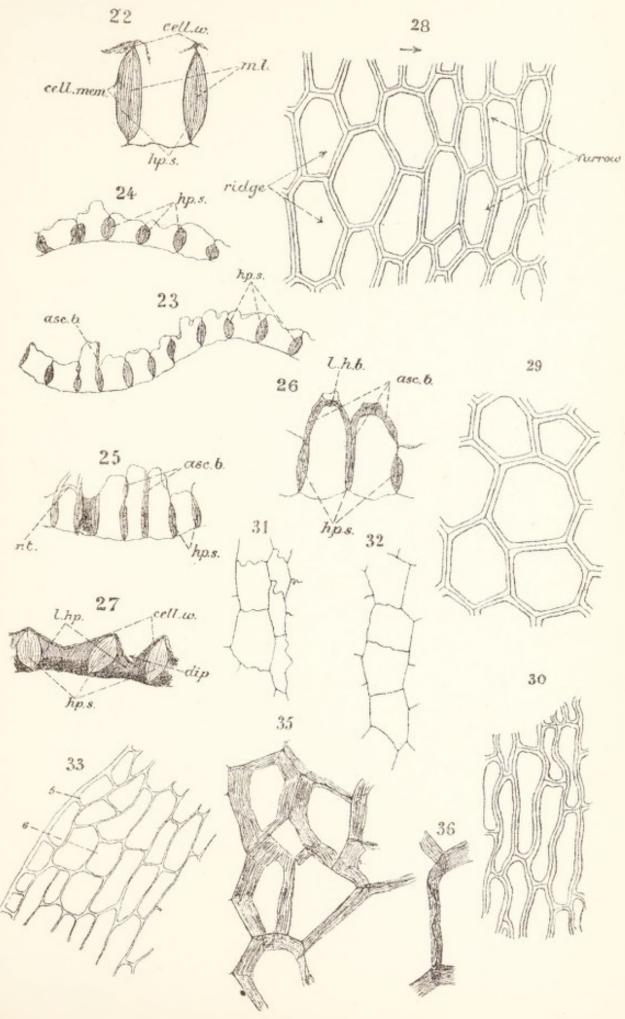




Plate VI.

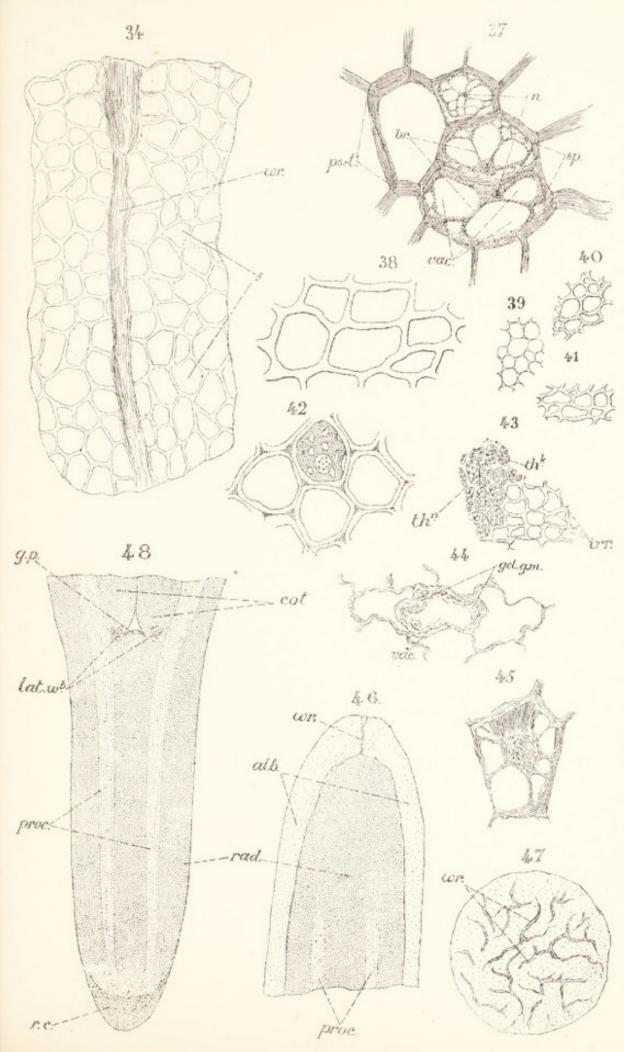




Plate VII.

