The Existence of Daily Growth-rings in the Cell Wall of Cotton Hairs.

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(Communicated by F. F. Blackman, F.R.S. Received February 25, 1919.)

[Plates 14–16.]

From studies of the growing cotton plant in Egypt* the author was led some years ago to the conclusions that the wall of the cotton-seed hair-cell was "probably composed of concentric layers, laid down during the active growth of each successive night, and numbering about twenty-five in all . . . they would thus, at the most, be about 0·0004 mm. in depth, so that their resolution by the microscope is highly improbable without some previous treatment."† Various methods were tried with the intention of bringing these layers into the limits of microscopic vision, but it was not until five years later that an accidental observation gave the clue to a method by which the limitations of microscope observation may be extended, and these layers made actually visible.

The observations which followed, demonstrating the existence of concentric layers in the wall of the cotton-hair as well as in the "fuzz-hairs," would have been interesting in any case on account of their bearing on all the physical and chemical problems which this typical cellulose presents. C. F. Cross has insisted on the necessity for considering cellulose problems in terms of "the ultimate fibre,"‡ but it now seems probable that the ultimate unit components must be the single layers composing the wall of the said fibre. The bare fact of the existence of such layers would have had no particular significance if it could not have been connected with previous precise study of the growth of cotton-hairs. By counting the number of layers in material previously preserved at known dates during the course of those studies, and remembering the cardinal fact that growth is daily arrested by the sunshine effect under Egyptian conditions,§ we have been able clearly to show that these layers are actually the growth-rings whose

* Summarised in 'The Cotton Plant in Egypt,' London, 1912; 'The Development and Properties of Raw Cotton,' London, 1915, p. 79; "Analyses of Agricultural Yield," 'Phil. Trans.,' B, 1915–17, Parts I–III.

† 'Raw Cotton,' p. 79.

‡ Presidential Address by C. F. Cross, Society Dyers and Colourists, 1918.

§ "The Physiology of the Cotton Plant," 'Cairo Sci. Jour.,' July, 1910, *et. seq.*

existence we had ventured to postulate. Knowledge of their real existence must materially affect some of our views concerning the physical properties of such hairs.*

Material.

The cotton material examined was chiefly of Egyptian origin, derived from my pure strain No. 77, on which most of the previous physiological work has been standardised, but samples from other Egyptian strains and varieties, together with cotton from other countries, have been used for check purposes.

In addition to mature cotton hairs (or "lint") and "fuzz," a small amount of material† pickled in acetic-absolute at all stages of growth had been brought by the writer from Egypt, amounting to 12 fruits in all. The growth-rings were shown by this equally well, in spite of five years and four months immersion in the 30 per cent. alcoholic solution of glacial acetic.

The seed-hairs of the cotton plant are of two kinds, the lint and the "fuzz." In certain wild cottons we find various groupings of abnormally long fuzz, short lint, or lack of differentiation between the two classes, which facts, in conjunction with the data from genetic studies of cultivated varieties, suggest that the two kinds of hair are intrinsically similar, in spite of the great difference between them in external appearance. In the case of pure strain No. 77, and most other Egyptian cottons, the fuzz-hairs are only about 1·5 mm. in length, clustered in two patches near the micropyle and base respectively of the seed, and exhibiting a vivid but unstable emerald green colour which fades to a rusty brown. Other cottons have fuzz which is devoid of colouring matter, while others again always show a brown colour without any precedent green. These colours occur also in the lint, a rare and little-known rogue strain of American (*G. hirsutum* ?) possessing vivid emerald green lint which quickly fades on exposure. The genetic peculiarities shown by various modifications, both of the seed-hairs proper, or lint, and of the subsidiary seed-hairs, or fuzz, are closely similar; this similarity extends not merely to colour-factors, but also to the distribution of each upon the seed-coat, and complex inheritance involving cryptomeres has been demonstrated in the latter respect for both by the writer.

The lint, or cotton fibre of common knowledge, is externally very unlike the fuzz, attaining a length of nearly 60 mm. in some varieties, and carrying a delicate brown colour at most, except in the green rogue above mentioned. The diameter of the embryonic fuzz-hair is nearly twice that of the lint-hair,

* Harrison, W., "Investigations on Textile Fibres," 'Roy. Soc. Proc.,' A, vol. 94, p. 460 (1918).

† 'Raw Cotton,' p. 175.

but this difference is less obvious when both are mature; the convolutions, which are characteristic of the lint, are not so well shown by the fuzz, on account of its thicker cell wall, but they are present, and, in sum, we may reasonably anticipate that any phenomenon shown by the fuzz may be expected to be found, in some modified form, amongst the lint-hairs as well.

Experimental Methods.

The first observation of these growth-rings was accidental, a laminated hair being noticed by the writer in some cotton treated by Cross and Bevan's method for the preparation of cellulose xanthate, with subsequent hydration.* The hair in question might well have originated from some source other than the cotton plant, but subsequent results have shown that it was a fuzz-hair. In any case, the phenomenon was so striking, and might prove to be so intimately related to the writer's previous researches in Egypt, that a systematic examination was undertaken, with the assistance of Dr. Mary Cunningham on the chemical side.

It was clear that the hydration process, or jelly formation, would need to be carried far enough to swell the wall of the cell to quite five times its initial thickness (fig. 1), and yet would have to be arrested some long way short of complete dissolution. In effecting this control we were able to take advantage of recent work by Cross and Bevan on the effects of CS_2 in conjunction with 9 per cent. NaOH (Engl. pat. 8342/18). At the best, however, we were unable to bring the process to complete certainty of demonstration in any one sample, so far as the growth-rings in the lint were concerned, though invariably successful with the fuzz. It seems evident that the attainment of the precise step at which the former are sufficiently swollen, and yet not too much, must be a matter of such careful chemical adjustment that the individuality of each hair may be concerned, and hence it must remain a matter of chance to a notable extent.† (Figs. 1, 6, and 7.) The point needs emphasis, for the sake of other workers.

* Cross and Bevan, 'Researches on Cellulose,' 1905–18 (Pat. 8700/92, etc.).

† [*Note added in Press, April* 28, 1919.—After various trials Dr. Cunningham has obtained preparations with cuprammonium (Schweizer's reagent) which show these rings as clearly as any made with CS_2 and NaOH, for occasional hairs only. It is of further interest that some of these preparations were made from cotton cellulose, deprived of cuticle, *e.g.*, fully bleached and subsequently boiled in solution of alkaline sodium sulphite. By comparing various hairs in these preparations it seems clear that cuprammonium usually contorts the growth-ring strata too much. Thus, fig. 63, in Mr. Matthews' book on 'Textile Fibres,' undoubtedly represents growth-rings thus deformed, as usual, beyond obvious recognition as such. I am indebted to Dr. Coward for bringing to my notice another example, though a more dubious one, of unconscious observation of these structures. W. Minajeff ('Ueber das erhöhte Anfarben de rmercerisierten Baumwolle und dessen Ursachen," in 'Zeitschrift für Farben-Industrie,' vol. 15, p. 234 (1907),

Both for lint and fuzz we finally settled on the following treatment:—a preliminary boil in 1 per cent. NaOH, followed by acidification with 1 per cent. acetic and washing; then, evacuation of the receiver with a Geryk pump, and injection with 9 per cent. NaOH *in vacuo;* some 3 c.c. of the soda having been used for 0·2 grm. of cotton, the receiver was opened, 3 c.c. of CS_2 added, and allowed to stand at room temperature. Samples which would swell up on wetting could usually be withdrawn within half an hour, although the reaction does not reach equilibrium until some hours have elapsed. After three or four days the hairs begin to revert, and the growth-rings are largely obliterated in the process.

Thus far we have not been able to make permanent microscope preparations, though slides mounted in water will keep for two or three days, and even show the rings in the lint upon being wetted after they have dried up.

The microscope objectives used were an old Swift's 1/3-inch and 1/6-inch, supplemented by Watson's Versalic 1/12-inch oil immersion. Fuzz rings can be recognised clearly with the first of these, and the presence of lint rings can be suspected by an indefinable appearance. Most of the phenomena described can be seen with the 1/6-inch, which gave a magnification of 250 diameters on the bench with the eyepiece used, while the 1/12-inch was chiefly employed to corroborate in counting the rings.

The illumination found most satisfactory for the lint rings was obtained from a nitrogen-filled lamp at a distance of 2 metres from the microscope, using the concave mirror and not employing the condenser substage; the substage diaphragm was kept wide open. The line drawings (figs. 8–11) were made with assistance from arc lamp projection and a prism, while for the fuzz photographs I am indebted to Messrs. Flatters and Garnett. The lint photographs were made by the writer with an extemporised apparatus. Most of the microscope equipment, owing to the impossibility of obtaining new apparatus at the time, hardly seemed adequate to the delicacy of the cytological problem.

The final magnifications obtained depended on the amount of swelling undergone by the wall, and in extreme cases the successive layers may be magnified as much as 20,000 times their estimated original thickness. This was made up of a forty-fold swelling and 500 diameters magnification with a 1/12-inch lens. The usual magnification, however, begins with a swelling of

figures four and five layers only in several adult hairs which had been swollen by cuprammonium. These appearances are described as fissures ("Ritzen") in the cellulose. While neither the description nor the drawings fit real growth-rings, it is possible that Minajeff may actually have seen them, but, not realising their nature, only noticed four or five out of two dozen.]

of five to tenfold, and with this amount there would seem to be no serious destruction of the cellulose structure.

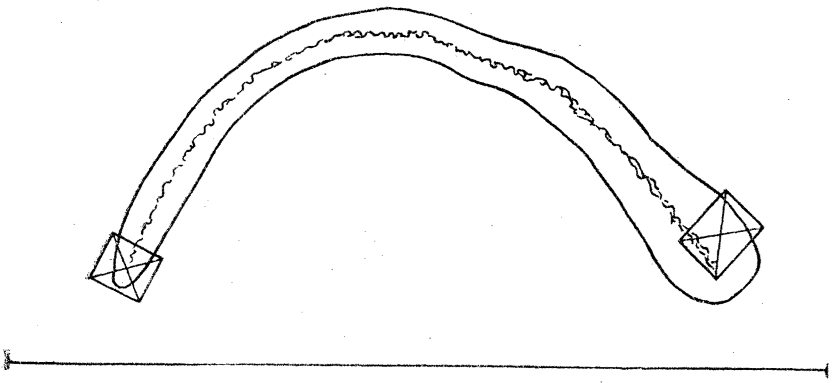

FIG. 8.

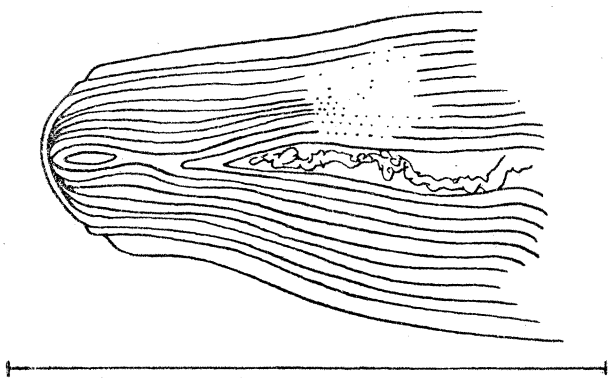

FIG. 9.

FIGS. 8 and 9.—Complete fuzz-hair swollen by treatment, and details of growth-rings in marked area at its tip. The two outermost rings broken away over actual tip. The isolated portion of the 10th ring could not be seen to be in connection with the main bulk of the ring.

Observations.

Intelligent understanding of organic structure must depend on knowledge of its growth-history, and it will therefore be more convenient to treat the observations from this point of view.

The differentiation of epidermal cells on the testa of the cotton-seed to form lint-hairs up to a length of 1 or 2 mm. was described by the writer in 1905.* Certain manipulative difficulties intervened to prevent further

* "The Sexuality of Cotton," 'Khedivial Agric. Soc. Year Book,' 1905.

study of the developing fruit, and no method was found until 1913, when the story was completely outlined* in connection with the physiological studies

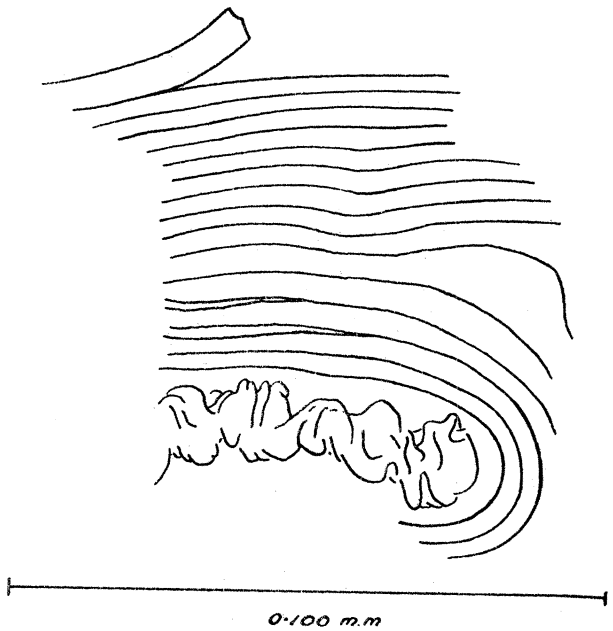

FIG. 10.—As preceding, but showing portion near base.

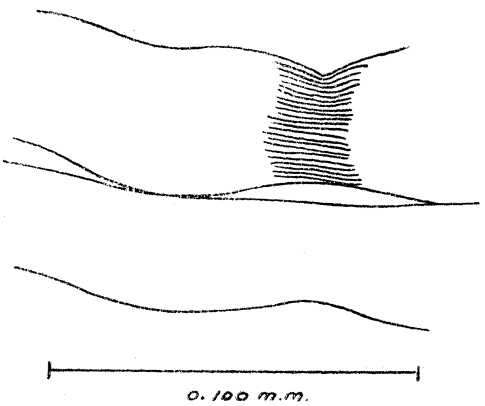

FIG. 11.—Adult lint-hair, showing swollen dimensions, and 23 rings as observed and repeatedly counted. Original diameter of cell lumen practically unchanged, *i.e.*, approximately 0·010 mm. on major diameter, with wall-thickness of 0·004 mm., making up a "ribbon-width" of 0·018 mm.

The wall-thickness of 0·004 mm. has been swollen to 0·030 mm.

* 'Raw Cotton,' pp. 80–85, etc.

of the process. The results were in no way peculiar on the cytological side, the hairs being simple cells with a large single nucleus and persistent cuticle, which grow to their full length during the first half of the maturation period of the fruit, and lay down secondary deposits of cellulose on their primary cuticularised wall during the latter half of the maturation period. For the purpose of these studies a large number of open flowers of my pure strain No. 77 were labelled on July 9, 1913, at the Giza Cotton Experiment Station, and a few were collected and pickled every three days until the fruits opened around the 50th day; a single boll from several stages was available for the present observations, of which the following are representative.

Nine-day Fruit.—The lint and fuzz are scarcely altered by the hydration process, and it is debatable whether any swelling of the wall has taken place at all. Judging by the proximity of the protoplasm to the cuticle at points where a hair had been accidentally bent, this swelling, if any, does not increase the wall thickness to more than 8 per cent. of the cell diameter, under parallel treatment with the well-swollen later fruits. Judging by the slight increase in visibility of the cuticle, such swelling may have happened, and this is supported by indications of wrinkling of the cuticle in surface view.

The primary cell wall can only contain a relatively small amount of cellulose, and probably the cuticle constitutes not less than one-third of its weight.

Twenty-one-day Fruit (fig. 2).—In spite of the great increase in lint length, which now approaches its adult value, the same description applies as for the nine-day specimen, with the exception of perhaps one hair in every few hundred, which is slightly swollen, so that the cell diameter is increased by about 30 per cent.

Twenty-seven-day Fruit (fig. 3).—Previous work had shown that the secondary thickening, with its concurrent formation of simple pits in the wall,* and the consequent ability of the hair to twist on drying, began round about the 21st–25th day.

Conformably with this we find that these hairs, under the xanthate treatment, present the typical beaded appearance. The great majority are swollen to three or four times the cell diameter, and the wall thickness in the swollen state may be equal to the cell diameter of the untreated hair. The remains of the cuticle, torn by this swelling but not themselves swollen, assume the position of girdles of various widths, or of spiral bands; both these formations are familiar as the result of treatment with cuprammonium solvent.†

* 'The Cotton Plant in Egypt,' p. 84, and 'Raw Cotton,' pp. 74, 77.

† O'Neill, C., 'Calico Printing and Dyeing,' London, 1862, and others.

At this stage, in hairs which had not swollen to the extent described above, very fine transverse wrinkling of the cuticle was seen, indicating that the transverse swelling of the secondary wall is accompanied by longitudinal contraction. This fact, which is indeed a matter of common experience with other reagents such as NaOH, propounds a question as to the space-dimensional internal structure of the cellulose wall, which has not been adequately answered.

The contraction is also demonstrated by the form which the remains of the protoplasm assume, since measurements show that this part of the hair is not appreciably swollen by the treatment, and yet it is evidently put under longitudinal compression. It follows that the cellulose wall in every degree of hydration, and presumbly, therefore, in its original untreated state, has some internal construction which is not the same in a tangential direction as is the case radially.

The swollen wall found in the great majority of the hairs at this stage is sometimes homogeneous, within the limits of microscopy, and sometimes shows a denser zone whereby the wall is divided into two portions. The outer of these two is the wider. No cases of more than two such portions have been noticed in the material examined, though it is probable that one or two more might be found exceptionally. Assuming that these layers might well be due to the growth of successive nights, and be demarcated from each other at the daily growth stoppage brought about by the sunshine effect, we may state that the average number of growth-rings at this stage is about 1·0; some hairs have two, while some have none. A variation of two days, plus or minus, in the dating of these events in the life history of the fruit is quite likely at this stage; the full maturation of the fruit in this strain has a P.E. of $\pm 3{\cdot}0$ per cent. which allows an extreme chance variation of $2\frac{1}{2}$ days either way at this present stage; in other words, the number of growth-rings may be expected to vary over a range of five, even if the individual hair-cells are no more variable than the massive tissue structure of the fruit.

Thirty-day Fruit.—The appearance of the fuzz-hairs at this stage is most strikingly different from that of their younger predecessors. The occasional dense layer seen in the swollen wall at 27 days is now multiplied, and these layers have become the most conspicuous part of the object, on account of their smooth curves. The first growth-ring is still quite twice the thickness of its successors; on tracing it to places where it passes under a girdle of cuticle there seems reason to believe that this extra thickness is not due to less constraint on its swelling, but that it was initially a thicker deposit of cellulose than its successors.

The lint-hairs also show lamination at this stage for the first time, but none have been observed on which dependable counts could be made.

Upon counting the number of growth-rings in the wall of 24 fuzz-hairs, the following frequency was obtained:—

Rings	0	1	2	3	4	5	6	7	
Number of hairs	0	2	3	8	5	3	1	2	= 24. Mean = 3·6

These figures are too small for any statistical generalisation, but it is evident that they vary only a little more than we anticipated, and that the mean is approximately three rings higher as the result of three days more growth.

Thirty-three-day Fruit (fig. 4).—The general appearance of this stage is like that of the previous one, but the count of growth-rings in the fuzz gave a mean value of 5·3, being 2·7 higher than the fruit of three days previously, with the following frequency distribution:—

Rings	0	1	2	3	4	5	6	7	8	9	10	11
Number of hairs	—	—	—	4	4	3	3	—	2	5	1	1

Photographs of one lint-hair showing ten rings, are reproduced in figs. 6 and 7, Plate 16.

Thirty-six-day Fruit (fig. 5).—The increase in the number of rings in this slide merely brought the value up to 6·6 on the first count taken, for reasons which will shortly be described. The frequency distribution was:—

Rings	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Number of hairs	0	0	0	1	1	4	1	4	5	5	3	2	0	0	0	0

The scatter of the observations is much the same as before, since 3 per cent. P.E. would allow seven days extreme variation at this age, against nine days actually observed, but the next two stages show that an observational error is creeping in.

Meanwhile we may note that four lint-hairs in this stage were in a state which enabled reliable counts of their growth-rings to be made, and were found to contain 7, 10, 11, and 12 rings respectively.

Thirty-nine-day Fruit.—The counts of the fuzz-rings were substantially identical with those from the previous stage, but the preparation was better

than the previous one as regards the lint, and seven hairs showed 10, 12, 13, 13, 13, 14, and 14 rings respectively. Subtracting the mean value, 13 rings, from 39 days, brings us back correctly to the 26th day as the one on which the first ring was formed.

Forty-two-day Fruit.—In this preparation the mean apparent number of fuzz-rings was again unchanged (7·2), though one very definite example of 14 rings was noted. No countable lint rings were found.

Mature, or Fifty-day Fruit.—The ripe fuzz and lint were examined in the pickled material, but also in various samples of lint and of seed, both of this No. 77 strain, and of others grown in Egypt and the Sudan. Less regular rings were found in some American Upland and Indian lints. The counts of rings in the lint could never be proved to exceed 25 in number, and the smallest figure obtained with certainty was 20, these numbers agreeing completely with expectation. Not more than 16 obvious rings have yet been seen in the adult fuzz, but it was presently found that the very decided line of demarcation, which the older growth-rings of the fuzz display, is prone to become less definite in the later, innermost rings. Further search showed that in some cases this decrease in definition was progressive after the first three or four rings, while in others it was quite sudden, and occasionally it was possible, in lucky preparations, to see clearly that the innermost "ring"—as casual observation had judged it to be—was actually compound (fig. 5 shows traces) and actually consisted of seven or even more rings which were indistinguishable from those shown by the lint.

This accounts for the apparent deviation of the number of rings in the fuzz from expectation based on our hypothesis. It does not exclude the possibility, in all the other cases where the evidence was negative, that the fuzz ceases to thicken its wall at an earlier date than does the lint, but the trend of the facts is evidently opposed to the latter assumption, and it is more reasonable to assume that the growth of secondary thickening in the fuzz is less "inhibited" at first than in the lint. The converse inhibition would seem to obtain as regards growth in length.

It is a striking fact, well in line with all the data from genetics and from the ordinary cytology of development, that the fuzz-hairs should degrade to the production of walls resembling those of the lint-hairs, when they are in a senescent condition,* and not before. The problems of cell-senescence in the cotton plant would seem to provide a most promising field for research.

* "Temperature and Growth," 'Ann. Bot.,' p. 557 (1908); 'Raw Cotton,' pp. 44, 96, 99, etc.

Anomalous Secondary Thickening of the Cell Wall.

While engaged in counting these rings in pickled material of known age, an abnormality was encountered under circumstances which indicate that two layers of cell wall may be formed simultaneously inside one and the same cell. Our observations thus link up with those of other workers on the sculpturing of spores on one hand, and with straightforward cell-physiology on the other.

It is not unusual to find in preparations made by the present method that actual lacunæ occur in the thickness of the wall, due to the cellulose of some one night's growth not having been "bonded" firmly to that of the previous morning. It is not difficult to imagine that this might result from various minute accidental causes.

In one hair, however, the writer observed a phenomenon for which he has found no exact parallel in cytological literature, though it suggests comparison with the behaviour of plasmolysed cells, and may have some relation to the debated subject of the growth of the sculptured exine on the surface of pollen-grains.

This hair was noticeable with a low magnification, having one very conspicuous growth-ring, seen in optical longitudinal section as a line in the wall on both sides, running from the base of the hair to a distance of 2 mm. along it. The whole of the hair could not be seen, but a length of 6 mm. was measured, and in all respects—except this conspicuous ring—it was a normal lint-hair. With higher magnifications it was found to be in such condition of hydration near its base that ordinary growth-rings could not only be seen, but counted, in several places. Most unfortunately, however, this state of preparation did not extend more than 1·5 mm. along the hair, so that no rings could be counted at the point where the conspicuous one, which had been obvious under the low power, died away. This conspicuous one appeared to be a surface of actual cleavage, though a recognisable lacuna was nowhere clearly visible, and at first it appeared to be merely an extreme case of the lacunæ already mentioned.

On counting the growth-rings, inside and outside this surface of cleavage, which we were able to effect in three separate places, the number of rings outside the cleavage was eight, while inside it there were either 13 or 14. The material in which this hair was found, came from a 42-day fruit; we have seen that the expected number of growth-rings at this stage is only 16, whereas we found 21 or 22. The highest number found in the 39-day fruit was 14, which in three days more becomes 17. The excess number of rings in this abnormal hair, above expectation, is almost in excess of the limit of

expectation. Also we have found that scarcely any hairs had begun to form their first rings on the 21st day, so we have either to assume that this hair was extremely early, as well as being abnormal otherwise, or else that we have here a case of dual wall-formation for the first eight days.

The writer personally inclines to the latter opinion, though the probabilities can just, and only just, be strained to bring the case into category of normal wall-formation; it seems therefore desirable to put this interesting observation on record.

Conclusions.

The following facts appear to be firmly established as the result of combining these observations with antecedent studies:—that the primary wall of the seed-hairs in cotton contains very small amounts of cellulose; that the secondary thickening of the wall proceeds intermittently under normal Egyptian field crop conditions, being arrested each afternoon; that the cellulose of the hair consequently consists of a number of concentric shells, layers, or "growth-rings," each one representing one day's growth, with the exception of that of the primary wall; and that the so-called fuzz-hairs are analogous with the lint-hairs, though their growth-rings are coarser and more sharply demarcated. The hairs are covered, outside the cellulose of the primary wall, by a cuticle, bearing wax, which is structurally and historically identical with the cuticle of the testa, while it is structurally and chemically distinct from the cellulose. The secondary wall, but not the primary, is traversed obliquely to the hair axis by simple pits which are rarely visible except in the living hair, and to these pits is due the twisting of the hair and its characteristic convolutions after death.

In the method employed we now possess a simple kind of ultra-microscopy, applicable to cellulose. The dimensions of the wall of the lint-hair are such that the thickness of each of the 25 growth-rings composing it can only be, at most, about 0·4 μ, much less than the wave-length of sodium light.

Summary.

The present communication describes the structure of the cellulose wall of the cotton hair, in relation to its development, as a continuation of observations previously published in "The Development and Properties of Raw Cotton."

By suitably swelling the cellulose-wall to some five or ten times its initial thickness, under treatments with CS_2 and NaOH, concentric layering becomes visible in the swollen walls.

Material of known age and development, fixed in Egypt, was examined by this method and it was found that there is only one thin primary layer while the hair is growing in length, but, that as soon as thickening of the wall sets

in, the number of layers increases day by day up to a maximum of 25, when thickening is complete.

As growth is arrested by sunshine in the middle of each day in Egypt, the number of these layers corresponded to the number of nights during which the growth in thickness of the cell-wall had continued.

These concentric layers in the wall of Egyptian cotton-hairs are thus rings of nightly growth, differentiated by heterogeneity like the annual rings in timber.

These rings are coarser, more sharply demarcated, and less regular, in fuzz-hairs than in lint-hairs. The later rings in fuzz-hairs may resemble those of lint-hairs.

Certain abnormal appearances indicate that the cellulose wall may grow for a time without being in full contact with the ectoplasm.

I have to acknowledge much valuable assistance in our laboratory from Dr. Mary Cunningham in the adjustment, application and interpretation of the chemical treatment, and from Mr. C. F. Cross, F.R.S., in directing my attention to the chemical problems of cotton.

My thanks are also due to the Fine Cotton Spinners' and Doublers' Association, Ltd., of Manchester, for permission to publish this note, and for having made possible the continuation, extension and application of my previous researches.

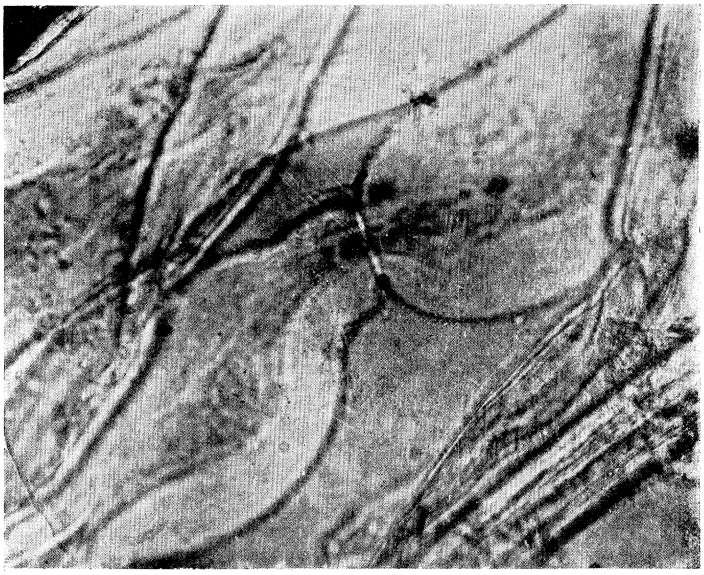

FIG. 1.

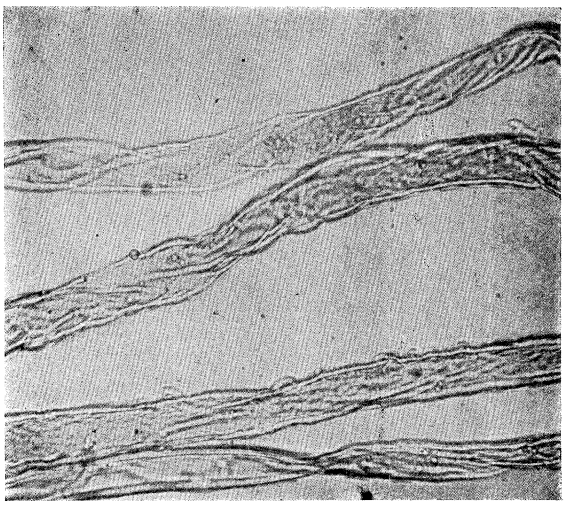

FIG. 2.

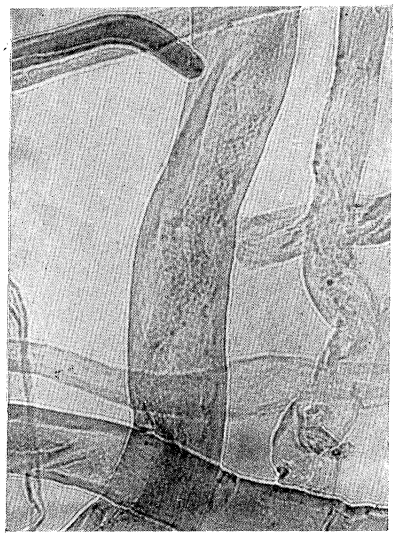

FIG. 3.

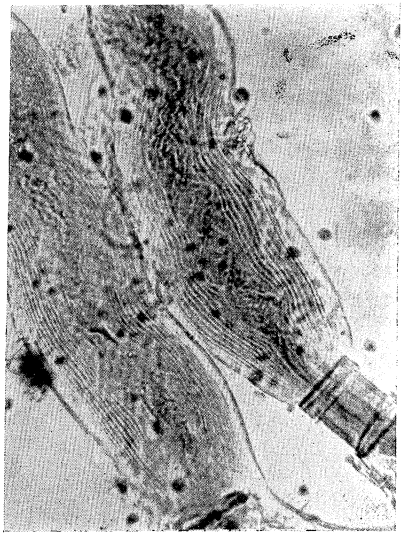

FIG. 4.

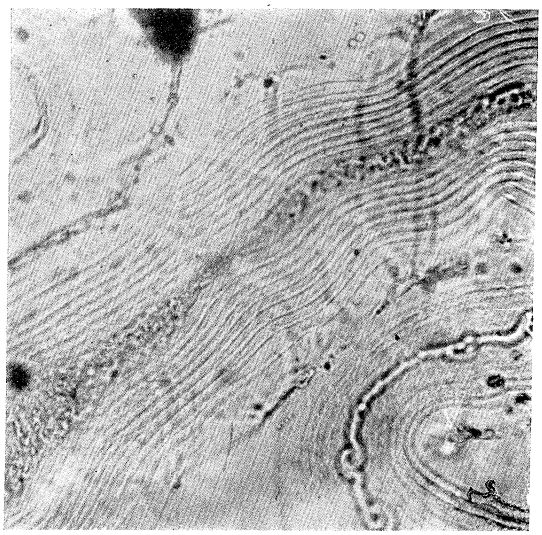

FIG. 5.

FIG. 6.

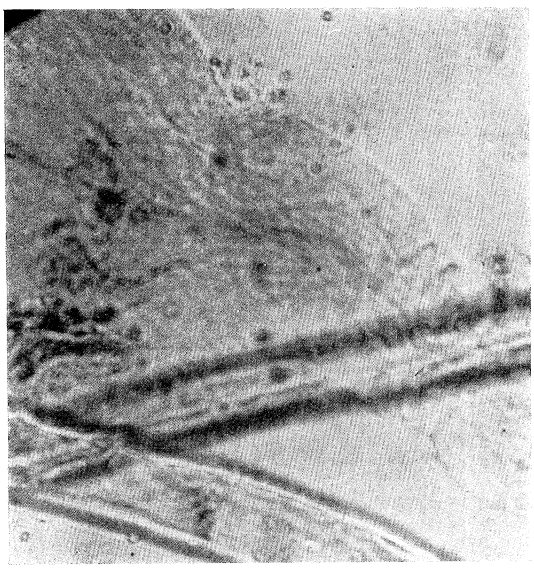

FIG. 7.

DESCRIPTION OF PLATES.

(All figures represent hairs of the author's No. 77 Pure Strain of Egyptian Cotton. Acetic-absolute pickle, five years old.)

PLATE 14.

Fig. 1.—Lint-hairs swollen to varying extents under nominally uniform treatment by CS_2 and NaOH. A cuticle girdle clearly shown, indicating original diameter of swollen hair. × 540.

Fig. 2.—Fuzz-hairs on 21st day of development of fruit (boll). Primary wall scarcely swollen by treatment, wrinkled and flaccid as in all preceding 20 days. × 360.

PLATE 15.

Fig. 3.—Fuzz-hairs on 27th day, secondary thickening of wall having begun about 26th day. Two hairs well swollen, with only one growth-ring visible ; demarcation of secondary deposit from primary wall is indicated. × 360.

Fig. 4.—Fuzz-hairs on 33rd day. Fine example of a wide girdle of cuticle ; also faint spiral of same. Nine and ten growth-rings ; constriction in girdle clearly shown. Swelling of cell-diameter is about three-fold, of wall is about seven-fold. × 360.

Fig. 5.—Fuzz-hairs on 36th day. Cuticular remains very evident as spirals. Firmness of growth-layers in the jelly under bending stresses can be seen at corners of photograph.

Central hair happens to have same number of growth-rings as one in fig. 4 ; the great difference in appearance is thus merely due to the greater swelling, which has magnified the cell-wall at least fifteen-fold. Magnification of growth-rings in this photograph is thus $15 \times 360 = 5000$ about.

PLATE 16.

Figs. 6 and 7.—Lint-hairs of 33rd day (for fuzz see fig. 4). Two successive photographs with minute alteration of focus ; original focussing effected directly on surface of a plate, not on ground glass. The two hairs practically unaffected by reagents indicate original dimensions of cell-wall. The swollen hair girdled by cuticular spirals passed into complete solution a little way outside the field of view ; maximum swelling in the field is about seventeen times the wall thickness. It is difficult to photograph the hyaline layers satisfactorily in optical section, but in various places the ten rings are indicated as a series of shadings. × 540.

Maximum magnification of growth-rings here is $17 \times 540 = 9000$ about.