THE DEVELOPMENT OF THE COTTON EMBRYO[1]

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INTRODUCTION

The development of the cotton embryo was treated briefly from the morphological point of view by Balls (*1*),[2] Flatters (*2*), and Gore (*4*). None of these investigators, however, made a chemical study of the developing embryo. Ordinarily such investigations have not been made because of the impracticability of separating the immature embryos from the surrounding tissues in sufficient quantities to make satisfactory analyses. Instead of the usual chemical analyses it is often practical to use microchemical tests, for, although they have the disadvantage of not yielding quantitative results, they have the advantage of being applicable to plant tissues in their natural position and to comparatively small quantities of material.

This study of the development of the cotton embryo was undertaken for the purpose of obtaining a knowledge of the rate of growth, anatomical development, and chemical development, as related to each other and to the age of the embryo from the first division of the zygote to dormancy.

METHODS

In July 1934 approximately 150 flowers of an American upland cotton (Startex) were self-pollinated. As the time after pollination that is required for fertilization to occur has been reported by Gore (*4*) to be from 26 to 32 hours, the present report includes no studies to determine the length of this period, but it is assumed that fertilization occurred within 36 hours. Material was collected, killed in Licent's fluid, sectioned, and stained with haematoxylin or safranine. Collections of embryos were made at the following periods (days) after fertilization, allowing 36 hours for fertilization to be completed: 1, 2, 3, 4, 6, 9, 12, 15, 16, 18, 22, and 26. Before the twenty-sixth day most of the important anatomical developments had occurred, and the oldest of the material was not used for anatomical studies. However, microchemical tests and growth studies were made on the older material.

Some difficulty was experienced in making microchemical studies on fresh material before the sixth day. This was caused by the minuteness of the embryo and the fact that it gave the same reactions as the endosperm surrounding it.

Weights of embryos 16 days of age and older were taken immediately after their dissection from the ovules, and again after drying in air for 24 hours. The lengths of embryos were recorded from the sixth day until maturity.

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[2] Reference is made by number (italic) to Literature Cited, p. 944.

EARLY DEVELOPMENT OF THE EMBRYO

The first division of the zygote, which ordinarily occurred the second day after fertilization, was usually horizontal (fig. 1, *A*). This was followed by a vertical division of the apical and sometimes of the basal cell (fig. 1, *B*). Occasionally a diagonal division instead of the vertical occurred in the apical cell, giving rise to such a structure as that shown in figure 1, *C*. The exact manner of origin of this configuration was not determined because of its relatively rare occurrence. A second departure from the condition shown in figure 1, *B*, occurred when the basal cell failed to divide (fig. 1, *C*, *D*), resulting in a one-celled suspensor. Three-celled proembryos were most frequently found in ovules killed the second day after fertilization.

An intermediary tier of cells was found (fig. 1, *K*), but never a single intermediary cell, as was reported in *Malva rotundifolia* by Souèges (*6*). A perfect quadrant was probably formed, and various slight departures from it were common. A group of three cells at the apex (fig. 1, *E*) also was found. The structure shown in figure 1, *E*, occurred in material killed the third day after fertilization.

A short suspensor was formed, but in some cases it showed early signs of disorganization. The latter fact may explain Balls' (1) statement that the cotton embryo has no suspensor.

In material taken the fourth day it was found that octants (fig. 1, *H*) had been produced from the quadrants by further cell division. About the same amount of irregularity was observed in the octants as had been found in the quadrants. Material taken the same day also showed mitoses and periclinal walls separating off the dermatogen (fig. 1, *G* and *I*).

Although the stages found here are irregular, if the succession shown in figure 1, *A*, *B*, *D*, *F*, *H*, and *J* is followed, the history of the development of the cotton embryo will be seen to be similar to that described by Souèges (*6*) in *Malva rotundifolia*. He gave the following account: The first division is horizontal and is followed by a vertical division. The bipartition of the elements of the tetrad gives an octocellular proembryo possessing four circumaxial cells in its upper part. Sometimes 1 of the 2 juxtaposed elements of the tetrad is segmented horizontally or obliquely. The 4 quadrant cells at the apex are separated by walls which take insertion on the peripheral membrane and come down to the vicinity of the axis on the lower horizontal wall (prenant insertion sur la membrane périphérique et venant tomber au voisinage de l'axe sur la paroi horizontale inférieure). Each of the 8 cells then divides, giving rise to a 16-celled proembryo. The wall of segmentation of the quadrant is often horizontal. Five tiers of cells are produced that give rise wholly or in part to cotyledon, hypocotyl and initials of the central cylinder, cortex at the summit of the radial central portion of the tip, and suspensor. This description of the embryogeny of *Malva rotundifolia* appears to be the only record in the literature relative to the early stages of the developing embryo in any malvaceous plant other than cotton.

A great deal of irregularity was found in the details of the early stages of development in the cotton embryo; however, the stages of development illustrated were studied carefully and to all appearances are natural. No doubt the various embryos were sometimes observed from different sides, but if they were radially symmetrical and regular

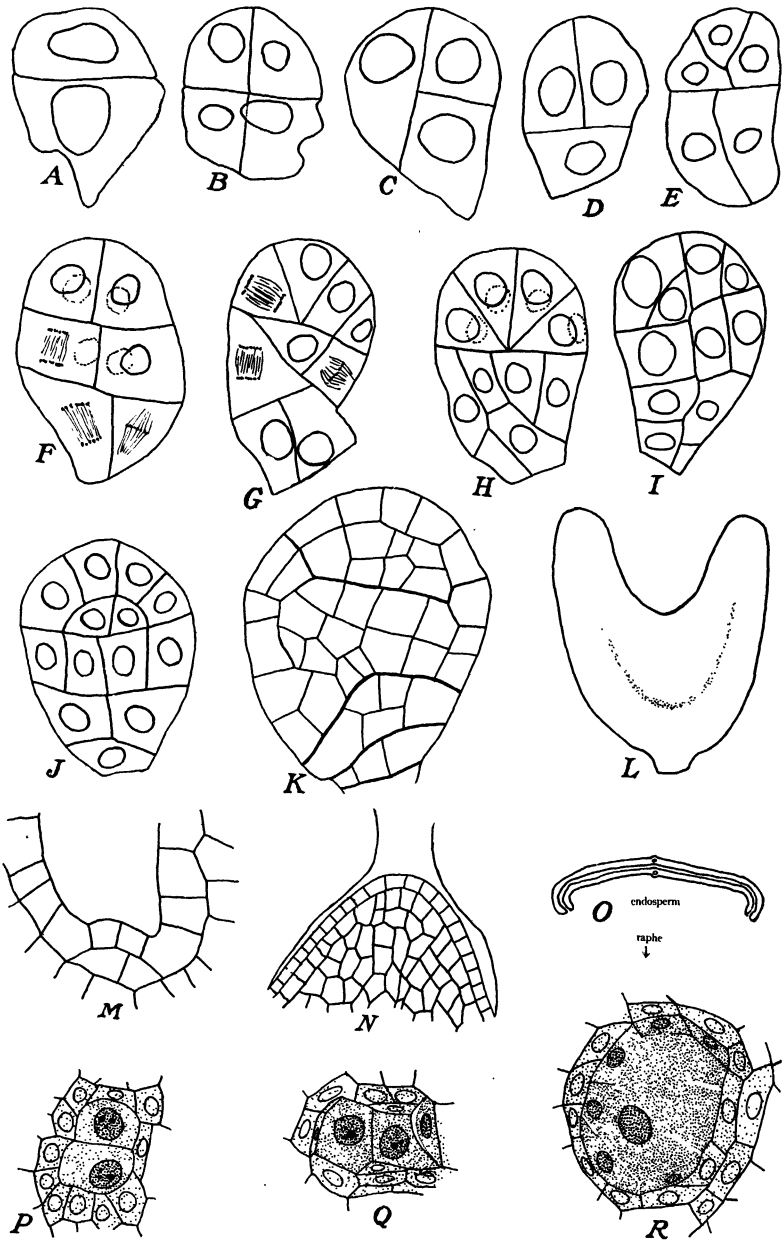

FIGURE 1.—*A–K*, Stages in development of the cotton proembryo and embryo in approximate succession, including irregular conditions, × 550, *L*, 9-day-old embryo showing early differentiation of cotyledons, hypocotyl, and histogens, × 123; *M*, apex of a 9-day-old embryo in a slightly later stage of development, showing the beginning of the plumule, × 550; *N*, plumule of a 15-day-old embryo, × 240; *O*, cross section of cotyledons of a 12-day-old embryo, showing their position with reference to the endosperm and raphe × 20; *P–R*, successive stages in the development of resin glands, × 550.

in early development, as proembryos are often thought to be, their appearance would always be much the same.

Because of the irregularities noted it is impossible to state the exact age at which cotton embryos reach any particular stage of development, but the data given throughout this paper probably represent the conditions that occur most frequently at the ages indicated.

The first microchemical tests were made when the embryo was 6 days of age. At this time, carbohydrates (Molisch reaction) and proteins were present. Negative results were obtained, however, when tests were made for starch, pentosans, and oils. The endosperm, which was very abundant at this age, gave exactly the same reactions as the embryo.

THE GRAND PERIOD OF DEVELOPMENT

The early development of the cotton embryo, as described above, took place before the rapid increase in size of the embryo had begun. About 9 days was required for the initials of the main organs, such as cotyledons, hypocotyl, and plumule, to be formed. The embryo then entered a period of rapid growth, and details of structure continued to manifest themselves as development proceeded. Organization of the cotyledons began between the sixth and ninth day (fig. 1, *L*), when the embryos became heart-shaped in general outline with the two auricles developing into cotyledons and the ventricle end into a hypocotyl.[3] Soon after the cotyledons had assumed their form a small mound of tissue could be observed in the axil between them (fig. 1, *M*). This was the first appearance of the plumule as such. The plumule did not become conspicuous until the fifteenth day (fig. 1, *N*).

Between the sixth and ninth day, the histogens of the hypocotyl made their appearance. The limits of the dermatogen, periblem, and plerome were recognized without difficulty on the ninth day. In material of this age proteins and starch were found to be present and pentosans and oil were absent. Reactions in the endosperm indicated a slight amount of starch, but otherwise the same materials were detected there as in the embryo. At 12 days of age the embryos averaged about 1.6 mm in length, and had taken a position on the side of the embryo sac approximately opposite the raphe (fig. 1, *O*).

In embryos 12 days of age the cotyledons averaged about 7 layers of cells in thickness, these layers being the upper and lower epidermis and 5 layers of mesophyll. The cells were fairly uniform in size and shape and often were in regular rows. As the embryos advanced in age the cotyledons increased in thickness by an increase in size and number of cells. At 12 to 15 days of age the earliest signs of provascular strands were found in the cotyledons. These signs appeared first through the formation of cell walls horizontal to the surfaces of the cotyledon, and later by walls vertical to these surfaces and longitudinal to the axis of the provascular strands. Differentiation of the palisade cells of the cotyledons had begun, also, at this age, the second layer of cells from the upper surface being 50 to 100 percent longer than the other mesophyll cells.

[3] The term "hypocotyl" is used in this paper in the broader sense to include all the organs of the embryo below the attachment of the cotyledons.

In 15-day-old material several other changes were apparent, the most interesting being the first signs of the resin glands. The first indication of a gland was the appearance in the cotyledon of a few (usually 2 or 3) large cells whose protoplasm was very granular and stained rather darkly (fig. 1, *P*). The surrounding cells were of ordinary size and shape except that they were slightly flattened. As the embryo advanced in development, the large cells increased in size, and the cells surrounding them became more flattened (fig. 1, *Q*). Signs of disorganization of the protoplasts of the large cells were obvious on the sixteenth day, for the nuclei had lost their identity, cell walls had begun to disappear, and the contents of the cells had assumed a still more granular, but otherwise homogeneous, appearance. A few layers of the surrounding flat cells also became disorganized and the cells surrounding them in their turn became flat and showed signs of disorganization (fig. 1, *R*). At this age the young glands were well on their way in development, and no structural changes were found between this condition and maturity, except a continuation of the phenomena already described.

Microchemical tests made on embryos 16 days of age showed traces of oil but no pentosans. Reactions of protein with biuret and Millon reagents were more intense in the embryo than in the endosperm, and this difference was observed until the seed was mature.

In embryos 18 days of age, the contents of the young resin glands gave the same color reaction with sulphuric acid that had been obtained in glands of mature embryos. Marchlewski (*5*) attributed this reaction in the mature embryos to the presence of gossypol. This test is by no means specific for gossypol, but when carefully used it is a fair indication of that substance. Sulphuric acid will produce a red color in other parts of the embryo, but the particular shade of color and the streaming of the gland contents observed at this stage of development are identical only with those obtained in the glands of mature embryos. It may well be emphasized here that the reactions indicating gossypol were not observed at the time of the first appearance of the glands, but only in embryos 18 days old or older. Gallup (*3*), in his studies of the time of appearance of gossypol in cottonseeds found gossypol first in 32-day-old seeds. His analyses were made on the entire ovule, rather than on the embryo alone as was done in the present investigation. Naturally, gossypol would be relatively less abundant in the entire ovule, and this may account for his not finding it in the young ovules where it had not become plentiful.

A study of 18-day-old material showed that pentosans had appeared in the glands and that the amount of oil in the cotyledons had greatly increased. Starch and proteins were still abundant in both the embryo and endosperm; and since it is well known that proteins, oil, and pentosans are common in fully mature embryos, tests for them were discontinued at 18 days after fertilization. Starch, however, was known to practically disappear before maturity, and in order to determine the time of its disappearance, tests were made for it until this condition was found. Starch seemed to disappear so gradually that the time when it began to decrease was not determined. It was found to be scarce at 33 days after fertilization; and traces were rarely found in seeds that were apparently mature. When the embryo was 18 days of age, all of the organs and provascular tissues were well on their way in development. All important chemical components of

the embryo identified in this study had also begun to appear, and most of them were fairly abundant. The remainder of the period of development of the embryo must therefore consist chiefly of changes (usually increases) in the amounts of the various components and increases in the size of the organs and in the size of the embryo as a whole.

Microchemical tests for sugar in the embryos were never positive except possibly with the Molisch and the Fehling tests. Tests for sugars were made not only on the embryo but also on young lint hairs and the seed coat. The results of these tests are given in table 1. From this table it may be seen that glucose is present in the developing lint hairs. The results were checked and found identical with results obtained on known corresponding substances. Although tests for glucose, except in the form of glucosides, conducted by other investigators on all parts of the cottonseed have consistently given negative results, there can be no doubt that the results obtained in this study on the young lint hairs are typical glucose reactions. In addition to the results summarized in table 1, tests for glucose were made on lint hairs soon after their appearance and also in the later stages of development. The results showed that glucose is an important component of the lint hairs from the time of their first appearance until the boll begins to open. The occurrence of glucose in these hairs is not surprising in view of the fact that the hairs are composed chiefly of cellulose and that a close relationship is known to exist between glucose and cellulose.

Positive reactions for glucose and fructose were obtained on the seed coat during its development, although these results were somewhat masked by a flocculent precipitate of some other component. After the reactions had been carefully studied and the tests repeated on filtered extracts of the seed coats, it was concluded that this structure contained substances that gave the reactions of fructose and glucose. The glucose reaction was not as strong as that of fructose.

TABLE 1.—*Summarized results of microchemical tests for sugars in the cottonseed coat, embryo, and lint hairs 10 to 26 days after fertilization*

Test	Lint hairs	Seed coat	Embryo
Phenylhydrazine	Positive, glucose	Positive? glucose	Negative.
Flückiger	do	Positive? glucose and fructose	Negative?
Fehling	Positive	Positive	Positive?
Molisch	do	do	Do.

Embryos gave the Molisch reaction for carboyhdrates from their early formation to maturity; however, the Flückiger and phenylhydrazine tests showed that this was not the result of the presence of glucose or fructose. Several other sugars and glucosides are known to occur in mature cotton embryos, and probably one of them was the substance that gave the reaction for carbohydrates. Indeed, starch, which is known to be present in developing embryos, gives positive reactions with the Molisch reagent, but it usually reacts more slowly than the carbohydrate under investigation here. The Fehling test showed a somewhat doubtful reaction for reducing sugars. A precipitate occurred, but its appearance was not typical of the copper oxide that precipitates when the test is applied to any of the common sugars.

THE CLOSING PERIOD OF GROWTH

In the closing period of growth of the embryo there were fewer changes than in the grand period. Storage materials continued to form, so that there was a slow increase in dry weight, but there was a great loss of water and therefore a loss in total weight. No new tissues or organs appeared and no new chemical components were recognized.

THE GROWTH CURVE

For several decades biologists have recognized the fact that the normal growth curve of an organism takes the general form of a somewhat straightened S; that is, growth begins slowly (the formative period), later becomes much more rapid (the grand period), and then again becomes slow preparatory to cessation (the maturation period). In the life of the sporophyte of a cotton plant, there are at least two such growth curves. The first represents the development of the embryo from the zygote to the mature seed in the resting period, and the second represents the time from the awakening of the embryo from its dormant period until the sporophyte has reached maturity. In the cotton embryo, the exact form of the growth curve (figs. 2 and 3) was found to vary in some details, depending upon whether the size of the embryo was recorded as length, immediate weight, or air-dry weight. The curves are very similar, however, and the differences are easily explained. It was not found practical to weigh embryos until 16 days after fertilization, but measurements of length were taken as early as 6 days after fertilization. The embryo entered upon its grand period of development in length about a week before it entered this period of development in weight. This is explained by the fact that during early development length increases more rapidly than volume. On the fifteenth day the embryos were approximately 3.8 mm long. Then they began to expand laterally, filling the cavity of the growing seed coat. From that time until the seed reached its greatest length, the weight and length curves were very similar; however, when this period was reached, about the twenty-sixth day after fertilization, the weights continued to increase at the same rate. The last 3 days of this period make up the period of maturation. The embryos continued to increase in actual size (live weight) until the thirty-fourth or thirty-fifth day, when they began to lose weight by drying. They

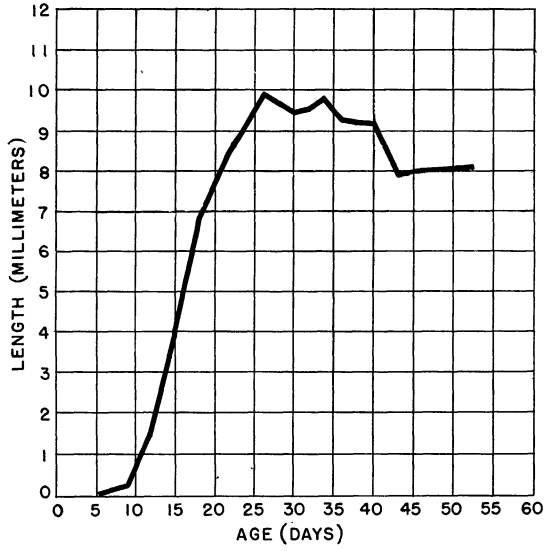

FIGURE 2.—Length of cotton embryos at different ages.

reached an equilibrium between the forty-third and forty-seventh day and then remained constant. The period of maturation in live weight occupied from the thirtieth to the thirty-fourth day. In air-dry weight, the embryos increased steadily until the thirty-second day and then entered upon the period of maturation. They attained their greatest weight on the fortieth day, although the variation observed in the curve after the thirty-sixth day was probably caused by the smallness of the samples. The curves based on weights show the period of maturation more adequately than the curve based on length. Gallup (*3*) states that the weight of the embryo may be expected to decrease slightly after maturity, possibly through respiration. A decrease would naturally occur in any seed, but the amount of decrease would be negligible.

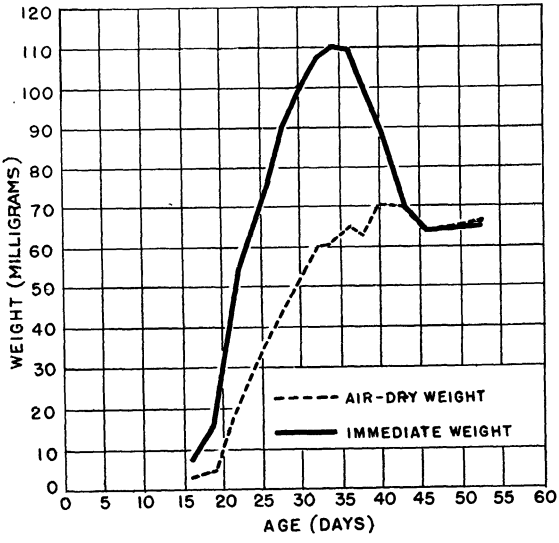

FIGURE 3.—Weight of cotton embryos at different ages.

The principal events observed in the developing embyos with their corresponding ages are summarized in table 2.

TABLE 2.—*Age in days of cotton embryos with corresponding morphological and chemical changes, green weight, dry weight, and length*

Morphological and chemical changes	Age	Embryos weighed	Green weight	Dry weight	Embryos measured	Length
	Days	*Number*	*Mg*	*Mg*	*Number*	*Mm*
Occasionally first division of zygote	1					
First or second division of zygote	2					
Quadrant and other similar stages	3					
Octant; separation of dermatogen	4					
Carbohydrates (Molisch reaction) and proteins present	6				2	0.07
Early organization of cotyledons and hypocotyl; appearance of histogens and starch	9				2	0.29
Location of embryo at side of embryo sac opposite raphe	12				15	1.6
Appearance in cotyledons of provascular strands, resin glands, and palisade tissue; early differentiation of plumule, appearance of oil	15				16	3.8
	16	20	7.5	3.0		
	18				13	6.8
Appearance of gossypol and pentosans	19	14	16.4	4.6		
	22	14	53.6	21.0	5	8.6
	26	20	77.8	38.0	12	9.9
	30	55	100.2	52.0	30	9.5
	32	14	107.1	60.0	29	9.6
Continuation of growth and differentiation of tissues that had already arisen	34	20	110.3	61.2	20	9.8
	36	59	109.8	64.7	31	9.3
	38	76	99.4	63.0	76	9.2
	40	50	90.6	70.2	50	9.2
	43	44	70.2	69.5	44	7.9
	46	30	63.5	63.3	30	8.0
	53	20	65.7	65.9	20	8.1

ABNORMALITIES AND IRREGULARITIES

Although the material used in this study was thought to be a relatively uniform race of American upland cotton, much irregularity was observed in the size of embryos of identical ages.

A few examples will suffice to show the range in size of embryos of the same age. Three 29-day-old bolls were taken at random, embryos were dissected from them, and the average live weights were found to be as follows: Boll 1, 20 embryos averaged 72 mg; boll 2, 20 embryos averaged 111 mg; and boll 3, 15 embryos averaged 123 mg.

After the bolls had been dried in air until an equilibrium was reached, the following average weights were recorded: Boll 1, 20 embryos averaged 33 mg; boll 2, 20 embryos averaged 60 mg; and boll 3, 15 embryos averaged 63 mg. It should be emphasized that these figures are the averages of 15 or 20 embryos, taken at random from the boll, and that the variation among individual seeds was probably much greater than that shown here.

Among the embryos from a single 19-day-old boll the range in length was from 3.5 to 7 mm. If this range were stated in volume or weight it would be much greater. In a fully open, 53-day-old boll, the range in length of 10 embryos taken at random was from 6 to 9 mm. This was an ordinary sized boll having 5 good locks and 37 seeds.

Such cases of variation were not at all uncommon, although it cannot be said that they were of regular occurrence. Whenever so wide a range of variation occurred it was usually in part the result of the fact that a few embryos were much smaller than others. For example, in the last case cited above, the individual measurements in millimeters were as follows: 6, 7.5, 8, 8, 8, 8, 8, 8.5, 8.5, and 9. Numerous ovules of all ages were found which at first appeared to be entirely devoid of embryos, but which upon close examination showed small, sickly ones.

Ovules that showed no embryo sacs, or very abnormal ones, were found occasionally. Undeveloped ovules caused by a lack of fertilization were common, of course, but were not among the irregularities studied. Observations indicated that the position of the seed in the locule was related to its ultimate size. Position is undoubtedly related to shape of embryo and therefore to length. Rea (7) obtained some evidence that motes, or abortive ovules, are caused by a lack of fertilization. Some of the irregularities reported here are undoubtedly related to motes, and further embryological studies should reveal useful information as to how they originate.

SUMMARY

A study was made of the anatomical and chemical development of the cotton embryo in relation to its rate of growth.

The early anatomical development was found to be somewhat irregular, but it showed certain similarities to the early embryonic development of *Malva rotundifolia* as reported by Souèges.

Details of the development of the resin glands were studied. Indications of gossypol were found much earlier in the development of these glands than had been previously reported.

Most of the organs and tissues began their development during the latter part of the formative period and the first part of the grand period of growth. On or before the eighteenth day, oil, starch, pentosans, gossypol, and proteins were formed. All of these materials, except starch, were found throughout the remainder of the growth period.

Glucose was not clearly demonstrated in the embryo at any time during the entire period of development, but it was found in the young lint hairs from the time of their first appearance until just before maturity.

A high degree of variation in rate of growth and in size of embryos within the same boll was found, in spite of the fact that the material was thought to be relatively pure and the flowers had been self-fertilized. Numerous irregularities in form were observed in mature embryos.

LITERATURE CITED

(1) BALLS, W. L.
1905. THE SEXUALITY OF COTTON. Khedivia Agr. Soc. Yearbook 1905: [199]–222, illus.

(2) FLATTERS, A.
1906. THE COTTON PLANT: ITS DEVELOPMENT AND STRUCTURE AND THE EVOLUTION AND STRUCTURE OF THE COTTON FIBRE. 92 pp., illus. London.

(3) GALLUP, W. D.
1927. THE GOSSYPOL CONTENT AND CHEMICAL COMPOSITION OF COTTON-SEEDS DURING CERTAIN PERIODS OF DEVELOPMENT. Jour. Agr. Research 34: 987–992.

(4) GORE, U. R.
1932. DEVELOPMENT OF THE FEMALE GAMETOPHYTE AND EMBRYO IN COTTON. Amer. Jour. Bot. 19: 795–807, illus.

(5) MARCHLEWSKI, L.
1899. GOSSYPOL, EIN BESTANDTHEIL DER BAUMWOLLSAMEN. Jour. Prakt. Chem. (9N. F.) 60: 84–90.

(6) SOUÈGES, R.
1922. EMBRYOGÉNIE DES MALVACÉES. DÉVELOPPEMENT DE L'EMBRYON CHEZ LE MALVA ROTUNDIFOLIA L. Compt. Rend. Acad. Sci. [Paris] 175: 1435–1436, illus.

(7) REA, H. E.
1928. LOCATION OF "MOTES" IN THE UPLAND COTTON LOCK. Jour. Amer. Soc. Agron. 20: 1064–1068, illus.