The Nucleolus and Nuclear Division in the Root-Apex of *Phaseolus*.

BY

HAROLD WAGER.

With Plate V

THE great prominence of the nucleolus in nearly all cell-nuclei, its definite form, its avidity for aniline dyes, and its behaviour during nuclear division led, more than twenty years ago, to the conclusion of Strasburger, Flemming, Guignard, and others that it is in some way connected with the growth and increased stainability of the chromosomes during the division of the nucleus. This view is still maintained by many cytologists, although the micro-chemical researches of Zacharias, and the later investigations of Strasburger are opposed to it.

From a series of observations which I have made upon the changes which take place in the nucleolus during the process of nuclear division in the root-cells of *Phaseolus*, it appears to me that not only is the nucleolus concerned in the formation of the chromosomes, but that there is a definite morphological connexion between them. Briefly stated, it is found that the nucleolus is intimately connected with the nuclear reticulum; that it contains nearly all the chromatin of the nucleus; that this is transferred, previous to division, into the nuclear thread, which is then segmented into chromosomes; and that, in the reconstitution of the daughter-nuclei, the chromosomes become fused into a number of more or less spherical or irregular masses which unite to form the daughter-nucleoli¹.

Although there are numerous observations which indicate a close relationship between nucleoli and chromosomes, the existence of such a definite morphological connexion between them had only been previously observed in a few cases, of which *Spirogyra* among plants and *Actinosphaerium* among animals are the most prominent examples. More recently a somewhat similar conclusion has been arrived at in the case of the root-cells of *Vicia*.

¹ These results were stated in a Paper read before Section K at the Bradford meeting of the British Association in 1900. See Brit. Ass. Reports, p. 944.

LITERATURE.

In the following brief account of the literature, reference is only made to the more important papers in which the relations of the nucleolus to the chromatin and chromosomes are dealt with, and more especially to those papers published since 1897. An excellent summary of the whole of the literature on nucleoli, down to 1897, is given by Montgomery in his valuable memoir on the Morphology of the Nucleolus¹.

According to Flemming² the nucleolus is a special organ of the nucleus or cell for the collection or elaboration of chromatin. It does not actually consist of chromatin (or nuclein), but of a substance in which the chromatin is elaborated, or, it may be, of a homogeneous substance which may be a chemical modification of chromatin or a preliminary stage in its formation. It possesses a definite form, but with no membrane around it, and it may be vacuolate with fluid contents in the vacuoles.

Strasburger³ regarded the nucleolus as an inert mass consisting of a reserve substance or substances, allied to chromatin, for the formation of the chromosomes. During division the nucleoli become dissolved in the nuclear sap and are then taken up into the nuclear thread, to reappear again in the chromatin-network of the daughter-nuclei.

Pfitzner⁴, Guignard⁵, Went⁶, and others give expression to similar views.

Strasburger, in his more recent memoirs⁷, adopts a different view from that just stated. He now believes that the chromatin is contained in the nuclear sap, and that one must look for its origin in the cytoplasm and not in the nucleolus. The nucleolar substance serves for the building up of the spindle. The evidence for this appears to be the complete or partial disappearance of the nucleolus immediately preceding the formation of the spindle; the active and quantitative condition of the kinoplasm rises or sinks as the nucleolus dissolves or reappears, and the solution of the nucleolus is followed by the highest point of spindle-development. He brings forward the observations of Němec, who states that the kinoplasm is directly transformed into nucleoli, and that granules which show the peculiarities of nucleoli arise at the poles of the division-figure through transformation of the spindle-threads. Their appearance is especially due

¹ Jour. of Morph. xv, 1899.

² Zellsubstanz, Kern- und Zelltheilung. Leipzig, 1882.

³ Ueber den Theilungsvorgang der Zellkerne und das Verhältniss der Kerntheilung zur Zelltheilung, Arch. f. mikr. Anat., xxi, 1882. Die Controversen der indirekten Kerntheilung, Arch. f. mikr. Anat., xxiii, 1884.

⁴ Beiträge zur Lehre vom Bau des Zellkerns und seinen Theilungserscheinungen, Arch. f. mikr. Anat., xxii, 1883.

⁵ Nouvelles recherches, &c., Ann. Sci. Nat., Bot., Sér. 6, xx, 1885.

⁶ Beobachtungen über Kern- und Zelltheilung, Ber. d. deutsch. Bot. Gesell., v, 1887.

⁷ Karyokinetische Probleme, Jahr. f. wiss. Bot., xxviii, 1895. Ueber Reduktionstheilung, Spindelbildung, Centrosomen und Cilienbildner im Pflanzenreich. Jena, 1900.

to a lower temperature, and Strasburger remarks that Hottes¹ has shown that a low temperature promotes the appearance of extranuclear nucleoli whilst a higher temperature promotes the formation of the spindle-figure.

Zacharias², in a series of important papers in which the structure and micro-chemical reactions of the nucleolus are dealt with, contends that by the action of digestive fluid nucleoli are clearly differentiated chemically from chromosomes, and that this difference is also brought out by staining in methyl green in which the chromosomes stain more deeply than the nucleoli. Nucleoli may be vesicular and may exhibit a differentiation of structure into a homogeneous peripheral portion and a more refringent, granular or vesicular central portion. They appear to consist of plastin with albuminoids, but contain no chromatin. By observations on the living cell, especially the rhizoids of *Chara*, he finds that the nucleoli disappear just as the nucleus is about to divide, and that, just before this happens, they undergo amoeboid changes of form. Several nucleoli appear in the daughter-nuclei, and these afterwards fuse together into one. No definite conclusions as to what becomes of the nucleolus during division, or its function can be stated. In the nucleolus of *Spirogyra*, which many observers maintain is morphologically connected with the formation of the chromosomes, he finds no chromatin; but he remarks in his most recent paper that could such a morphological connexion between nucleolus and chromosomes be established it would follow from the combination of morphological and chemical data that during the formation of the elements of the nuclear plate definite chemical changes would take place.

Carnoy's observations³ led him to distinguish four kinds of nucleoli—(1) 'nucléoles nucléiniens,' (2) 'nucléoles-noyaux,' (3) 'nucléoles plasmatiques,' and (4) 'nucléoles mixtes,' a combination of (1) and (3). He thinks that the plasmatic nucleoli may be concerned in the formation of the spindle. The 'nucléoles nucléiniens' are part of the chromatin-network and may consist of an amorphous mass of chromatin or of a chromatin-thread.

Macfarlane, in his earlier contributions⁴ and in his latest⁵ paper, contends that the nucleolus is the most specialized portion of the cell and contains the main mass of the chromatin-substance.

¹ Reduktionstheilung, Spindelbildung, Centrosomen und Cilienbildner im Pflanzenreich, p. 127.

² Zacharias, E., Ueber den Zellkern, Bot. Zeit., xl, 1882. Ueber den Nucleolus, Bot. Zeit., xliii, 1885. Ueber das Verhalten des Zellkerns in wachsenden Zellen, Flora, lxxxi, 1895. Ueber einige mikrochemische Untersuchungsmethoden, Ber. d. deut. Bot. Gesell., xiv, 1896. Ueber Nachweis und Vorkommen von Nuclein, Ber. d. deut. Bot. Gesell., xvi, 1898. Ueber die achromatischen Bestandtheile des Zellkerns, Ber. d. deut. Bot. Gesell., xx, 1902.

³ La biologie cellulaire. Étude comparée de la cellule dans les deux règnes. Lierre, 1884. La cytodierèse chez les Arthropodes. La Cellule, i, 1885.

⁴ Trans. Bot. Soc. Edinburgh, xiv, 1881. Trans. Royal Soc., Edinburgh, xxx, 1885, and xxxvii, 1892.

⁵ Current Problems in Plant Cytology. Contributions from the Botanical Laboratory, University of Pennsylvania, ii, 1901.

Mann¹ considers it probable that the nucleus and nucleolus are concerned in the assimilation of food-material, and that the nuclear chromatin may be less highly elaborated material than the nucleolar chromatin. He suggests that the nucleolus may be either 'an organ for the further transformation of substances already elaborated by the nucleus, or simply a storehouse for food-material, which has been already transformed by the nucleus into substances directly available for the nourishment of the achromatic elements of the cell.'

In *Spirogyra*, according to Tangl², Meunier³, Moll⁴, Decagny⁵, Henneguy⁶, Mitzkewitsch⁷, and Van Wisselingh⁸, the nucleolus contains chromatin and the chromosomes are derived entirely or in part from it.

Moll points out that only the nucleoli retain gentian violet with obstinacy; from all other parts it is extracted without difficulty. The nucleoli are found in three different forms—(1) homogeneous, (2) vesicular, (3) exhibiting a skein structure. The last is the more common. Chromatic substance does not exist to an appreciable amount outside the nucleolus. The nuclear segments are formed by the transference of the chromatic substance from the nucleolus into the nuclear plasm. 'It seems as if the chromatic substance were squeezed from the nucleolus by an aperture' into the nuclear plasm in which it appears 'as small fragments, ranged in an intermediate, achromatic thread, like the beads of a necklace; and thus a skein, containing chromatic substance, is formed.'

Mitzkewitsch states that in the process of division the nucleolus increases in size, its membrane disappears and it becomes irregular in shape. It then becomes differentiated into a number of deeply stained granular chromosomes, which form the nuclear plate, and a less deeply stained substance in contact with them. In the daughter-nuclei the chromosomes can be distinguished still surrounded by this less deeply stained substance, with which they eventually become incorporated to form the daughter-nucleoli.

A somewhat different account is given by Van Wisselingh, who believes that two only of the chromosomes are derived from the nucleolus, the others being derived from the nuclear thread. In the reconstitution

¹ The Embryo-sac of *Myosurus minimus*, L. A Cell Study, Trans. and Proc. Bot. Soc. Edinburgh, 1892. See also Brit. Ass. Reports, 1892, p. 753.

² Ueber die Theilung der Kerne in *Spirogyra*-Zellen. Sitz. d. k. Akad. d. Wiss. in Wien, lxxxv, 1882, p. 268.

³ Le nucléole des *Spirogyra*. La Cellule, 1888.

⁴ Observations on Karyokinesis in *Spirogyra*. Verhand. der k. Akad. van Wetenschappen te Amsterdam, 1893.

⁵ Bull. Soc. Bot. France, xlii, 1895, p. 319.

⁶ Leçons sur la Cellule, 1896.

⁷ Ueber die Kerntheilung bei *Spirogyra*. Flora, lxxxv, 1893, p. 81.

⁸ Ueber den Nucleolus von *Spirogyra*: ein Beitrag zur Kenntniss der Karyokinese. Bot. Zeit., lv, 1898, p. 195, and Flora, lxxxvii, 1900.

of the daughter-nuclei the halves of these two chromosomes again give rise to the new nucleoli.

Whatever may be the exact method of division which takes place, it seems clear, as Strasburger says¹, that we have here a special behaviour of the nucleolus in giving rise to the chromosomes which, although opposed by Zacharias on micro-chemical grounds, appears to be morphologically decisive.

According to Golenkin² we have a somewhat similar phenomenon in *Sphaeroplea*. The nucleolus breaks up into a number of fragments, which arrange themselves in a nuclear disk, and then appear to split up and move to the two poles where they fuse into daughter-nucleoli. All the chromosomes appear to originate from the nucleolus. He says that similar nuclei occur in other green Algae, including all Volvocineae, and in Musci.

Rosen³ states that in some plant-cells two kinds of nucleoli may be present, 'Eunucleoli' and 'Pseudonucleoli.' The Pseudonucleoli form part of the chromatin-network, and are used up in the formation of the chromosomes. The Eunucleoli are like the ordinary nucleoli, and do not disappear until a later stage in nuclear division. In the root-apex of *Phaseolus* there is only one kind of nucleolus present, which becomes lobular in the pro-phases of division, and does not entirely disappear in some cases until the separation of the daughter-groups of chromosomes is completed.

Macallum⁴ has shown that some nucleoli give an intense reaction for iron. This indicates that they contain chromatin. He finds in *Erythronium* at least three kinds of nucleoli:—(1) nucleoli which give a weak reaction for iron, and which therefore contain little or no chromatin; (2) nucleoli rich in iron, and which give a reaction for iron in every respect like the nuclear thread; (3) nucleoli found in the embryo-sac. The nucleoli of the embryo-sac appear in the filaments during the retrogressive stage as spherical elements containing little iron. As the chromatin in the filament becomes reduced they give a stronger reaction for iron, and eventually are found to consist chiefly of chromatin, 'and in stained preparations appear to contain nearly all the chromatin of the nucleus.' 'When mitosis again commences the filament forms at their expense, the increase in size of the filament keeping pace, apparently, with the decrease in the quantity of chromatin which the nucleoli contain.'

V. Häcker considers⁵ that nucleoli are not to be regarded as reserve

¹ Loc. cit., 1900.

² Bull. Soc. Imp. Nat. Moscou, 1899 (1900), p. 343. See J. R. M. S., 1901, p. 65.

³ Beiträge zur Kenntniss der Pflanzenzellen, Cohn's Beitr. z. Biol. d. Pflanzen, v, 1892, and vii, 1895.

⁴ On the Distribution of Assimilated Iron Compounds, other than Haemoglobin and Haematin, in Animal and Vegetable Cells, Q. J. M. S., N. S., xxxviii, 1895.

⁵ Praxis und Theorie der Zellen- und Befruchtungslehre. Jena, 1899. Die Vorstadien der Eireifung: zusammenfassende Untersuchungen über die Bildung der Vierergruppen und das Verhalten der Keimbläschen-Nucleolen. Arch. f. mikr. Anat., xlv, 1895, p. 200.

substance, but as products of excretion resulting from the metabolic change taking place between the cytoplasm and nucleus, and especially a secretion of the chromatin, which is thrown out of the nucleus during division.

Farmer¹ states that in the Liverworts he examined 'the nucleolus was associated with the chromosomes in an unmistakable and remarkable manner.' Threads of delicate texture run from the nucleolus to the linin. These are especially well seen in *Fegatella*. In a considerable number of cases the decrease of nucleolar substance is contemporaneous with the growth of the chromosomes. In *Fossombronia*, for example, 'the nucleolus becomes angular, and, in extreme cases, almost star-shaped, whilst at the same time the linin begins to exhibit a very striking increase in the amount of chromatin which it contains.' In a later stage the very much distorted nucleolus is often connected with several of the chromosomes, and soon after disappears. In the daughter-nuclei the nucleoli appear early, first as two or three small bodies which finally fuse to one large one, and the linin concomitantly loses its chromatin constituent. This does not necessarily imply that the chromatin passes, as such, into the nucleolus. But some constituents of the chromatin may find their way into this body, since both chromatin and the nucleolus readily yield albumen on appropriate treatment.

Miss Ethel Sargent's observations on the formation of the sexual nuclei in *Lilium Martagon*² support the view that the nucleoli are concerned in chromosome-formation. The linin-thread during its growth appears to be fed from the partially dissolved nucleolus. Drops of nucleolar matter are found attached to the linin-thread in certain stages of its development. During the later stages of chromosome-formation the segments of the thread become shorter and thicker, and ultimately 'the colouring (staining) of the chromosome segments becomes uniform. Each is apparently homogeneous. There is no contrast between cyanophilous granules and erythrophilous ribbon, but the whole chromosome stains uniformly like chromatin.'

Miss Sargent has very kindly allowed me to examine her preparations of the embryo-sac nuclei, and I find that, as she and Professor Farmer³ point out, there is a definite relation between the linin and the nucleoli. Both in the resting stage and in the synapsis the nucleolus is connected to the linin-network by delicate threads, and in the later stages the chromosomes in process of formation are also often connected to it by threads.

Carnoy and Lebrun⁴ describe a series of complicated changes in the nucleus of the egg of the Batrachia from which it appears that the

¹ On Spore-Formation and Nuclear Division in the Hepaticae, Ann. Bot., ix, 1895, p. 469.

² The Formation of the Sexual Nuclei in *Lilium Martagon*, Ann. Bot., x, 1896 and xi, 1897.

³ Loc. cit. (Hepaticae), p. 491.

⁴ La cytodiérèse de l'œuf. La vésicule germinative et les globules polaires chez les Batraciens. La Cellule, xii, 1897, and xiv, 1898.

chromosomes are definitely derived from the nucleoli. The young nucleus contains a much convoluted, apparently continuous thread. A portion of this becomes transformed into nucleoli (primary nucleoli). The remainder becomes resolved into granules, some of which dissolve, and the others are converted into secondary nucleoli. The nucleus now contains only nucleoplasm and primary and secondary nucleoli. Both primary and secondary nucleoli become resolved into filaments which present very complicated figures. They are ephemeral, however, and again break up into granules, some of which contribute to the formation of new secondary nucleoli. These again produce new filamentous figures which are also ephemeral and from which comes a third generation of secondary nucleoli, and so on for a number of generations. All the filamentous figures found in the nucleus up to the time of the first polar kinesis have thus a nucleolar origin, and finally a portion of the products of this nucleolar resolution is dedicated to the formation of the nuclein elements (chromosomes) of the first maturation-spindle.

Montgomery¹ comes to the following conclusions concerning the structure and function of the nucleolus.

The ground-substance of the nucleolus is more or less dense, homogeneous, or granular, and either fluid or viscid in consistency. In *Spirogyra* it has a true membrane. Vacuoles are normal structures. The alveolar structure of nucleoli described by various observers (e.g. Cavara) is probably referable to the regular distribution of equal-sized vacuoles in the nucleolus. Nucleolini, granules within the nucleolus, have frequently been observed, but no particular morphological significance can be attached to them. They appear to be only detached portions of the nucleolar substance, and may in some cases be only small vacuoles.

Two kinds of nucleolus may be seen in some animal egg-cells, the nucleolus proper and the paranucleolus or Nebennucleolus. The paranucleolus usually stains less deeply with nucleolar stains. It is probably not present in plant-cells. Some observers consider the paranucleoli to be derivatives of the nucleolus. Hacker regards them as secretions of the chromatin. Montgomery considers that they may represent those portions of the nucleolar substance which are deposited last, after the nucleus has undergone important physiological and chemical changes, the first portion produced being the nucleolus proper. In some cases a double nucleolus is found, the component parts of which may each represent a true nucleolus; or such a double nucleolus may consist of a true nucleolus in apposition to a chromatin-nucleolus. The chromatin-nucleolus may be a metamorphosed chromosome (in *Pentatoma*), or, as in the larva of *Carpocapsa*, it may originate from one of the granules of the nuclear reticulum².

¹ Comparative cytological Studies, with especial regard to the Morphology of the Nucleolus, Jour. of Morph., xv, 1899, p. 265.

² Loc. cit., p. 519.

This seems to show that the chromatin-nucleolus always stands in genetic relation to the chromatin, whilst the nucleolus proper does not.

The mode of transportation of the nucleolar substance to the daughter-nucleoli is probably different in different objects. A discharge of nucleolar substance into the cytoplasm appears to take place in some cells and may disappear, hence 'the substance of the parent-nucleolus may be in many cases not transferred to the daughter-nuclei, but the latter (perhaps as a rule) may produce their own nucleoli *de novo*.' There is no substantial basis for Zimmermann's conclusion 'omnis nucleolus e nucleolo,' and there is no evidence that the nucleoli are genetically related to the chromatin.

The nucleolus 'is derived in the first place from the cytoplasm,' and consists 'of a substance, or different substances, taken into the nucleus from the cell-body.' 'It seems probable that these substances stand in some relation to the nutritive processes of the nucleus, and in a relation to the growth of the latter.' Thus growing nuclei are 'characterized by an especially large amount of nucleolar substance.' From this one might conclude that the nucleolar 'substance stands in some connexion with the processes of nutrition' and is either: (1) 'nutritive in function,' or (2) 'represents that portion of substances assimilated by the nucleus from which all nourishment has been extracted, and in this case it would be a waste product,' or (3) 'may represent accumulations of nutritive substance retained in the nucleus as a reserve supply; but this does not seem to be very probable, for by this assumption it would be difficult to explain the uniformity in the size of the nucleoli in a given species of cell.'

Cavara¹ has made an important contribution to this subject by his researches on the nucleoli of various plant-cells. I abstract briefly his chief conclusions. Nucleoli in a state of rest in cells still capable of division or growth are composed of two substances, one, internal, which forms the major part, a homogeneous and specially refractive substance, only slightly stainable, which corresponds to the plastin of Zacharias or pyrenin of Schwarz; the other, peripheral, of variable density, much more stainable than the plastin, with characteristics that connect it with chromatin or a modification of chromatin. These two substances are associated in various ways: sometimes the chromatin-like substance is uniformly distributed around the plastin, or more frequently is not so uniform, but presents breaks in its continuity which give to the nucleoli an alveolate structure or sometimes even a *real reticulate structure*. During the prophases of division the nucleoli decrease somewhat in volume and break up. At the same time the linin-thread contracts and breaks

¹ Intorno ad alcune strutture nucleari. Estratto dagli Atti del R. Istituto Botanico dell'Università di Pavia, nuova serie, v, 1898. Breve contribuzione alla conoscenza del nucleole. Bollettino della Società Botanica Italiana, 1902, p. 108. Bot. Cent., xxxix, 1902.

up into chromosomes. The nucleolar remnants which may remain in many cells, after the formation of the chromosomes, have no longer the characteristics of the nucleolus as presented before karyokinesis. They have become reduced in volume and in capacity for stains. This means that the chromatin-like substance has been subtracted from the nucleolus to build up the chromosomes, leaving the plastin which is employed in another manner, as in the formation of spindle-fibres and the new cell-wall. As the nucleoli are reconstructed during the anaphase, they become centres of attraction, not only for plastin, which is taken up from the nuclear sap, but also in part for chromatin, which is taken up from the nuclear thread. The differences which have been observed in the capacity of nucleoli for stains can be explained by the fact that they contain varying quantities of the two substances (see also Hertwig, loc. cit.). Without impugning the constancy of the characteristics of the nucleoli and chromosomes, 'non vi ha dubbio che gli uni e gli altri siano organi di una certa mutualità e transitorietà, e che come avviene dissoluzione di nucleoli o *pirenolisi* in seno al nucleo, altrettanto si possa dire di dissoluzione della cromatina, o di *cromatolisi*.' Chromatolysis ought not then to be regarded merely as an abnormal or pathological phenomenon, but it represents a normal condition, *sine qua non*, of the evolution of the cell.

Cavara's conclusions are combated by Longo¹, who says that from his observations the nucleolus is not formed of two distinct substances, and that the so-called plastin portion of the nucleolus represents only a vacuolization.

Buscalioni² also concludes that there is no connexion between the formation of the chromosomes and the disappearance of the nucleoli.

Bradley M. Davis³ shows that in the tetraspore mother-cells of *Corallina officinale* the chromatin is scattered in a finely divided state in the nucleus, and that a linin-network is wanting. Each nucleus contains a single nucleolus which stains differently from the chromatin-granules. After division the chromosomes fuse together into a large deeply stained body on the surface of the prominent centrospheres. A nuclear membrane is formed and then the nucleolus reappears, at first smaller than the chromatin body, but afterwards becoming larger. The chromatin body then begins to fragment into a number of minute granules surrounding the single conspicuous nucleolus.

¹ Existe cromatolisi nei nuclei normali vegetali? Rendiconti della R. Acc. dei Lincei, 7^a ser., v, 1898. Contribuzione alla cromatolisi (picnosi) nei nuclei vegetali. Estratto dal vol. 9^o degl' Ann. del R. Istit. Bot. di Roma.

² Osservazioni e ricerche sulla cellula vegetale. Ann. del R. Istit. Bot. di Roma, vii, 1898. See Bot. Cent., lxxix, 1899.

³ Kerntheilung in der Tetrasporenmutterzelle bei *Corallina officinalis*, L., var. *mediterranea*. Ber. d. deut. Bot. Gesell., xvi, 1898.

Macallum¹ shows that the reaction for phosphorus obtained in nuclei by means of his nitric-molybdate reagent indicates the presence of chromatin in the nucleoli. 'The eosinophilous nucleoli in animal and vegetable nuclei give a strong reaction for phosphorus, but less marked than in the case of chromatin. On the other hand, the nucleolar elements in the nucleus of the ovary of *Erythronium* which are rich in "masked" iron, give a deep reaction for phosphorus. A similar result was obtained in the nucleoli of the nuclei of the embryo-sac of the same form, in the peripheral nucleoli of the maturing ovarian ova of *Menobranthus*, in the nucleoli of *Corallorhiza multiflora* and of *Spirogyra*, all rich also in "masked" iron.'

A very interesting case, on the animal side, in which the nucleolus takes part in the chromosome-formation is that of *Actinosphaerium*. Here, according to Hertwig², the nucleolus, at one stage in the life-history of the organism, consists of plastin only (plastin-nucleolus); at another stage both plastin and chromatin are collected into a single homogeneous body, the plastin-chromatin-nucleolus. There is, however, no sharp distinction between them, the one passing gradually into the other so that the more chromatin the nucleolus contains the more it reacts like the chromatin-nucleolus, and vice versa. In mitosis the plastin-chromatin-nucleoli produce fine ramifying threads, which exhibit a granular appearance. The granules react like chromatin, whilst the ground-substance reacts as plastin. The equatorial plate is formed out of these threads. In the division of the primary cysts, which result in the formation of secondary cysts (gametes), the daughter-nuclei are reconstituted in such a manner that the chromatin remains distributed on the nuclear network, whilst the nucleolus is composed of plastin. In mitosis the chromosomes are formed out of the nuclear thread. As they become grouped to form the equatorial plate, the plastin-nucleoli become drawn out into fine granular filaments, which surround the chromosomes and become more or less incorporated with them. The spindle is formed out of the nuclear network and cytoplasm, but the connecting fibres between the two groups of daughter-chromosomes appear to be formed out of the plastin-substance of the nucleolus. The rudiments of the plastin-nucleoli appear as blisters in the chromatin masses, from which Hertwig suggests the possibility that both plastin and chromatin are modifications of, or represent the same element in, the nucleus.

According to Němec³, the nucleoli consist of a substance like plastin. The development of the spindle coincides with the disappearance of the

¹ On the Detection and Localization of Phosphorus in Animal and Vegetable Tissues, Proc. R. Soc., lxiii, 1898.

² Ueber Kernteilung, Richtungskörperbildung und Befruchtung von *Actinosphaerium Eichhorni*. Abh. bayer. Akad. d. Wiss., 1898. Ueber die Bedeutung der Nucleolen, Sitzungsber. Bot. Ges. München, xiv, 1898. See L'Année Biologique, iv, 1898.

³ Cytologická pozorování na vegetacích vrcholcích rostlin: Věstník král. České společnosti nauk Prag, xxiii, 1897 (see L'Année Biologique, iv, 1898); Zur Physiologie der Kern- und Zelltheilung, Separatabdruck aus Bot. Cent., lxxvii, 1899.

nucleolus. The mantle-fibres of the spindle condense during the anaphase into a granular mass, which becomes the nucleolus and passes into the daughter-nucleus. The extranuclear nucleoli which are sometimes seen at the anaphase along the new cell-wall also originate from the connecting fibres.

Némec, however, in a later paper¹, points out that, although his observations indicate the connexion between nucleoli and spindle-fibres, other explanations of the phenomena are possible, and remarks that these observations only prove certainly that there is some definite relation between the nucleolus and the division process, and that there is a definite connexion between the size of the nucleolus and the power of the nucleus to divide; in this he agrees with Schwarz². The solution of the nucleolus and the formation of the spindle-figure might be two quite different processes, having this only in common that they go on side by side.

Chamberlain³ finds a distinct connexion between nucleoli and chromatin in the oosphere-nucleus of *Pinus Laricio*. The chromatin takes the form of nucleoli, which collect from all parts of the nucleus to a definite area near the centre, and there develop into a typical spireme.

Duggar, in his studies on the pollen-grain and embryo-sac in *Symplocarpus*, *Peltandra*, and *Bignonia*⁴, has some very interesting observations on the relation of the nucleoli and the chromatin-elements. The nucleolus takes the chromatin-stain mainly, the nuclear reticulum very little. During the formation of the chromosomes they remain attached to the nucleolus by minute linin-threads, and the nucleolus is often drawn out by these into a fusiform shape. In *Bignonia*, 'in the dispirem of the daughter-nuclei the chromosomes become very irregular in outline, and gradually diminish in size, while there is gradually formed a large nucleolar-like body with irregular outlines. This body takes the chromatic dyes as did the nucleolus generally before.' Speaking of the second division, the author says: 'Everything indicates that the nucleolus of the microspore-nucleus has thus resulted from the direct or indirect fusion of chromatic material used in division.'

Mottier⁵ concludes that in *Dictyota* 'the behaviour of the nucleolus, during both the development of the nuclear figure and the construction of the daughter-nuclei, indicates that this body represents a substance which is utilized by the chromatin and not by the spindle-fibres.'

¹ Neue cytologische Untersuchungen : Sonderabdruck aus Fünfstück's Beiträgen zur wissenschaftl. Bot., iv, 1900.

² Die morphologische und chemische Zusammensetzung des pflanzlichen Protoplasmas. Cohn's Beitr. z. Biol. d. Pflanzen, v.

³ Oogenesis in *Pinus Laricio*, Bot. Gaz., xxvii, 1899.

⁴ On the Development of the Pollen-grain and the Embryo-sac in *Bignonia venusta*, Bull. Torrey Bot. Club, xxvi, 1899. Studies in the Development of the Pollen-grain in *Symplocarpus foetidus* and *Peltandra undulata*, Bot. Gaz., xxix, 1900.

⁵ Nuclear and Cell Division in *Dictyota dichotoma*, Ann. Bot., xiv, 1900.

Wiegand¹ points out that the nuclei in *Potamogeton* are all very peculiar, differing from the ordinary type in having the chromatin mostly aggregated in a ball at the centre of the cavity, instead of being distributed on the linin-network.

Andrews² shows that in the nuclei of *Magnolia* and *Liriodendron* the nucleolus is large, stains deeply, and has a conspicuous vacuole. The linin-network is chiefly connected with the nucleolus. The threads of the network are at first smooth and uniform in diameter. At a later stage granular masses of chromatin appear on it, which gradually increase in size and become the chromosomes. The nucleolus at about the same time disappears entirely. 'It is probably utilized as food in the growth of the chromatin masses, for they stain much more readily at this time than at an earlier stage.' The chromosomes therefore arise from the resting nucleus as irregular masses without a previous formation of the usual spirem, and their identity from the first to the second mitosis is not maintained.

Blanche Gardner³, on the root-cells of *Vicia Faba*, comes to the conclusion that the chromosomes derive 'at least a large part of their material from the nucleolus.' 'The nucleolus is related to the nuclear reticulum in such a way that the fibres penetrate its substance.' Previous to the nuclear division the nucleolus divides into two. Then the thin, long, almost continuous nuclear thread can be seen 'at one or at several points' to 'dip into the nucleolus.' 'The nucleolus now begins to transfer its contents into the nuclear thread.' The thread becomes thicker and stains just like the nucleolus, which gradually disappears. This spirem thread then divides transversely into the chromosomes. In the daughter-nuclei the chromosomes 'aggregate to form a small, dense, blue-black coil.' Out of this mass the nucleolus is formed. The chromosome-coil gives up its chromatin and gradually loses its dark colour. 'At first these chromosomes are full of the dark chromatin-granules; these become fewer and fewer as the nucleolus becomes larger and more distinct⁴.'

METHODS.

The root-apices of varieties of the common French Bean, *Phaseolus vulgaris*, L., were used. Sections were cut both by hand and by the microtome. Various fixing-fluids were used, but Perenyi's fixing-fluid was the most useful. Among the many staining methods tried, Heidenhain's iron

¹ Wiegand, Karl M., The Development of the Embryo-sac in some Monocotyledonous Plants, Bot. Gaz., xxx, 1900.

² Karyokinesis in *Magnolia* and *Liriodendron* with special reference to the Behaviour of the Chromosomes, Bot. Cent., xi, 1901, Beih., p. 134.

³ Studies on Growth and Cell-division in the Root of *Vicia Faba*. Contributions from the Botanical Laboratory, University of Pennsylvania, ii, 1901.

⁴ Chamberlain, in a recent paper (Bot. Gaz., xxxvi, 1903, p. 28) concludes that in *Pellia* the nucleolus contributes material to the chromosomes and spindle.

haematoxylin gave the best results. Other stains, however, were used for special purposes. Very careful staining and washing-out is necessary in order to exhibit the finer details of nuclear structure, and a good illumination with high powers is required for the microscopic examination of the preparations. The apochromatic object-glass of Zeiss (2 mm. N. A. 1.40) and an oil immersion condenser, or Powell and Lealand's dry apochromatic condenser (N. A. .98) were almost always employed; but a Leitz $\frac{1}{12}$ oil immersion lens was also found valuable. Daylight was often used for general work, but the light from an incandescent mantle passed through a bull's-eye condenser was used to determine the finer details of structure.

I am much indebted to my friend Mr. Norman Walker, Assistant-Lecturer in the Yorkshire College, who was kind enough to make some of the preparations for me.

THE RESTING NUCLEUS.

The resting nucleus does not appear to differ materially in structure from the ordinary nuclei of plant-cells. It is limited towards the cytoplasm by a thin deeply stained layer—the nuclear membrane—which exhibits in thin sections a fine granular structure. Inside this is a finely meshed nuclear network, which forms a thin layer at the periphery of the nucleus in close contact with the nuclear membrane. On it are distributed a number of small granules, which stain deeply in nuclear stains.

Each nucleus contains one or more nucleoli, which are usually much more conspicuous than the nuclear reticulum, and stain more deeply. The number present varies according to the age of the cell. It is only in the young cells, however, that two or more occur; as the cells come to maturity the nucleoli fuse together so that in all the older cells one nucleolus only is present. Each nucleolus is lodged in a cavity in the nuclear network, and is surrounded by a clear space. As in many other vegetable nuclei, it is suspended in this cavity by a number of delicate threads, radiating from it on all sides to the nuclear network (Pl. V, Figs. 3-7). These threads are only visible in stained specimens. They appear to be continuous with the nuclear network, and to form a part of it.

The relative size of the nucleolus and amount of nuclear network varies in different cells. In the actively growing and dividing cells of the meristematic region the nucleolus is the most prominent feature in the nucleus, the nuclear network forming only a very thin layer in close contact with, and scarcely distinguishable from, the nuclear membrane. In the cells of the root-cap the nucleolus is much smaller, but the nuclear network is relatively more abundant, and in the outermost older cells of the root-cap layer is in its turn the most prominent feature in the nucleus, the nucleoli being so small in some cases as to be scarcely visible. The same differences are

observable in other parts of the young root. It thus appears that the amount of nucleolar substance present in a nucleus is to some extent a measure of the nuclear activities.

STRUCTURE OF THE NUCLEOLUS.

The nucleolus is spherical in shape or nearly so, and is usually placed in or near the centre of the nucleus. It does not lie free in the nucleus, but, as already pointed out, is in close connexion with the nuclear network, in which it is suspended by fibres which not merely surround it, as Montgomery states (*loc. cit.*, p. 506), but actually penetrate its substance, and in many cases appear as if drawn out of it. The nucleolus in fact appears to form a part of the nuclear network. I have observed similar connecting fibres in the embryo-sac nuclei of *Lilium* in Miss Sargent's preparations, both in nuclei in the resting stage and in synapsis and later stages, in the apical cells of the stem of *Elodea*, in the root-cells of the Oak, and in many other cases. They have been observed by Farmer in the nuclei of Liverworts and *Lilium*; by Duggar (*loc. cit.*) in various plants; by Rosenberg in the nuclei of the suspensor of *Zostera marina*, L., and by other observers in various nuclei—both of animal and plant cells. Zimmermann's statement¹ that the nucleolus in no case appears to be in direct connexion with the chromatin-network is therefore not correct.

In some cases the nucleolus appears to be homogeneous throughout, but in the majority of nuclei it possesses one or more vacuolar spaces filled with a substance which appears to differ from the rest of the nucleolus (Figs. 1-7, 37). This vacuolization always appears to take place as the nucleus comes to maturity. It is absent only in young nucleoli. I have not seen the alveolar structure described by Cavara (*loc. cit.*), but it is obvious that the presence of a large number of vacuoles in a nucleolus would produce some such appearance as he describes.

In some of the larger nucleoli I have observed darker coloured granules (nucleolini) as described by Macfarlane (*loc. cit.*) and others, but I agree with Montgomery (*loc. cit.*) that no particular morphological significance can be attached to them at present. In the cases observed by me they seemed to form only the central portion of the vacuolar substance.

The vacuolate structure of nucleoli appears to be very common, and was first of all noticed in vegetable-cells by Schleiden, who speaks of the nucleolus as 'a small, sharply defined body, which, judging from the shadow that it casts, appears to represent a thick ring or hollow globule².'

Nägeli also observes that nucleoli may be homogeneous or 'hollow in

¹ Die Morphologie und Physiologie des pflanzlichen Zellkernes. Jena, 1896, p. 40.

² Contributions to Phytogenesis. Translated by Henry Smith in 1847 for the Sydenham Society, p. 234. See also Schleiden's Principles of Botany, Eng. Ed., translated by E. Lankester, 1849, p. 32.

the centre,' and that 'there may be one, two, three, or many cavities of various sizes and arrangement¹.'

Macfarlane² regarded the vacuoles as endonucleoli and ascribed to them an important part in the nuclear economy, and he still insists on their probable importance in living cells, and suggests that they play a 'special part in furnishing to the nucleolar substance some ferment or compound which may be utilized during the division period³.'

Mann⁴ regards the endonucleolus as 'the trophic centre for all the organs concerned in assimilation and dissimilation,' and says that it plays an important part in the conjugation of cells.

Cavara⁵ contends that the vacuoles indicate the separation of the nucleolar substance into two distinct parts, chromatin and plastin, and Zacharias, who is opposed to the chromatin-plastin theory of the nucleolus, nevertheless concludes that it appears to consist of two distinct substances, a more refringent vesicular substance surrounded by a homogeneous, less refractive substance⁶.

Chamberlain has described a case in which the central, less deeply stained portion appeared to be separable from the outer, more deeply stained peripheral part, for 'there seemed to be a crack in the nucleolus, and upon applying a gentle pressure the central portion came out of the shell⁷.'

In haematoxylin-stained specimens of *Phaseolus* it is very clearly seen that the outer layer becomes more deeply stained than the vacuolar substance; in some cases there is a very marked distinction between them, and in some nucleoli the vacuolar substance is in contact with the exterior through an opening in the outer layer on one side of the nucleolus (Fig. 5).

In methyl green and fuchsin the nucleolus stains bluish-red, the nuclear network red, and in specimens from which the stain has been well washed out the outer layer of the nucleolus is coloured light blue, the vacuolar portion remaining colourless or nearly so. Similar results are obtained with methyl green and eosin. In gentian violet the outer layer is deeply stained violet and the vacuolar portion light blue. But in many of the larger nucleoli the gentian violet shows up a more complex structure. In such cases we find a deeply stained thin outer layer surrounding a less deeply stained inner layer, and in the centre one or more deeply stained masses which are irregular in shape, with coarse radiations into the lighter stained part, or even exhibit a structure akin to a coarse network, which recalls the description given by Carnoy of his *nucléoles-noyaux*.

The existence of a chromatin-like substance in the nucleolus is indicated by the fact that in all these stains the chromosomes stain like the nucleolus, except that there may be a difference in the intensity of the

¹ Memoir on the Nuclei, Formation, and Growth of Vegetable Cells, translated from Schleiden und Nägeli's Zeit. f. wiss. Bot., 1844, by W. Henfrey, p. 239. Ray Society, 1845.

² Loc. cit., 1882-5.

³ Loc. cit., 1901, p. 194.

⁴ Loc. cit., 1892, p. 396.

⁵ Loc. cit.

⁶ Loc. cit., 1885.

⁷ Loc. cit., Bot. Gaz., 1899.

colouration. Thus in gentian violet and safranin the nucleolus stains red, the chromosomes deep red, whilst the cytoplasm and nuclear network are stained violet. In safranin and picro-nigrosin, well washed out, the larger part of the nucleolus stains deep red, but a thin peripheral layer stains blue, sometimes giving to the nucleolus the appearance of being surrounded by a membrane. The chromosomes stain light red or reddish-blue, the spindle deep blue, and the cytoplasm and nuclear network blue.

In gentian violet the chromosomes are usually more deeply stained than the nucleoli of the resting nucleus; but this appears to be due partly to the fact that the stain is more easily washed out of the nucleoli.

The chromatin-like substance thus appears to occur mainly in the outer more deeply stained portion of the nucleolus. This is further substantiated by the following observations.

In sections cut by hand from fresh root-apices and stained in dilute acetic acid solution of methyl green the protoplasm and nucleus both stain bluish-green, but the nucleolus is the most deeply stained, especially in the actively growing cells, and the outer layers of the vacuolate nucleoli are the most deeply stained. If fresh sections are placed in pepsin solution for from three to six hours, at a temperature of 36° C., a contraction and distinct diminution of the cell contents are shown. The nucleolus becomes much smaller and presents a bright glistening appearance. On staining in the methyl green solution, the protoplasmic remnant stains green, while the nucleolar remnant stains bright green, and in some cases a few granules, probably chromatin-granules in the nuclear network, take a similar bright green colour. The nuclear network stains like the cytoplasm.

Again, if sections, either fresh or in spirit, be treated according to the method of Macallum, we get a strong reaction for phosphorus in the nucleoli and chromosomes, but very little in the nuclear reticulum. In vacuolar nucleoli this reaction is confined mainly to the outer layers; we get very little in the vacuolar portion. And further, we get an intense reaction for phosphorus in the nucleolar remnant which is left after treatment with digestive fluid.

In gentian violet-stained specimens the nucleolar remnant left in contact with the chromosomes at the time of the formation of the nuclear plate (Fig. 21) is often much less deeply stained than the chromosomes, and in some cases probably represents that inner portion of the resting nucleolus which does not become stained deeply in nuclear stains. It is impossible to definitely decide this point, however, by microscopic examination.

The observations just described all point to the conclusion that there are at least two different substances in the nucleolus, one of which at any rate possesses the reaction of chromatin. It seems to me probable that, as Cavara suggests, the explanation of the different accounts which have been

¹ Loc. cit., 1898.

given of the structure and staining and chemical reactions, may be due to the fact that nucleoli at different stages of their development, or from different plants, may vary as to the relative amounts of plastin and chromatin substance which they contain. It seems almost certain that there are nucleoli which do not contain any chromatin or very little (the plasmasomes, true nucleoli, &c., of various observers), and others which contain a large quantity of chromatin (the so-called chromatin-nucleoli, *nucléoles-noyaux*, &c.), and between these two extremes we may have nucleoli with varying relative amounts of the two substances¹. Miss Ferguson mentions, in her paper on *Pinus Strobilus* (Ann. Bot., 1901, p. 433), that 'the occurrence of unstained nucleoli in the same nucleus in which others were deeply coloured was common, especially at about the time of synapsis.' Again, 'the attitude of this (egg) nucleolus towards dyes varies much at different periods of its history. It may or may not take the safranin of Flemming's triple combination; it may stain intensely with gentian violet or iron haematoxylin; it may show a weak reaction or may be absolutely unaffected by them.' It is not clear from the evidence available that the vacuolization of the nucleolus indicates a definite separation of the contents of the nucleolus into chromatin and plastin. It is more probable that the ground-substance of the nucleolus is plastin, and that the outer layer of it may become impregnated with chromatin or a modification of it.

CHANGES IN THE NUCLEOLUS DURING THE PROPHASE.

In the resting condition the nucleolus is suspended to the peripheral network by delicate threads, which are only visible in carefully stained specimens. As the nucleolus increases in size the suspending threads become more prominent, and it is then seen that they are intimately connected with the nucleolus, appearing as if drawn out of its substance, and on the other side are continuous with the peripheral network (Figs. 6 *a* and 7). In Fig. 6 *a* is seen a nucleolus at this stage which has become displaced from a nuclear cavity. The connexion of the threads with the nucleolus is clearly seen. In the root-apex of *Allium Cepa* Němec² points out that in younger cells the nucleoli are surrounded by a clear space, and are connected by achromatic fibres to the nuclear network. The hyaline appearance of this space may be simply due to the fact that chromatin-granules are absent from these threads. In somewhat older nuclei he points out that the radiating threads become more prominent, and chromatin-granules are now seen lying thickly on the surface of the nucleolus, and appearing as if fused with its outer layer. His figure illustrative of this stage corresponds almost entirely with my Fig. 6, for which I offer

¹ Cf. Montgomery, Woods Holl. Bio. Lectures, 1898.

² Ueber die karyokinetische Kerntheilung in der Wurzelspitze von *Allium Cepa*, Pringsh. Jahrb. f. wiss. Bot., xxxiii.

a different explanation, viz., that it indicates the beginning of the transference of nucleolar material to the nuclear thread ¹.

The peripheral network of the resting nucleus as seen from the surface is shown in Fig. 2. It consists of a lightly stained network with numerous small chromatin granules. It stains less deeply in nuclear stains than the nucleolus, and is not very conspicuous in the resting stage. At the time that the suspending threads become more prominent, certain portions of the peripheral network become also more prominent and more deeply stained (Fig. 9), a number of thicker threads being visible, connected to one another by finer filaments of the original network. The impression conveyed to an observer on looking through a large number of such stages in the nuclear development is that the substance of the nucleolus is being conveyed into the surrounding threads, and this is borne out by observation of later stages, where the threads radiating from the nucleolus become larger and more definite, and stain exactly like the nucleolus, which at the same time is becoming smaller and more irregular or amoeboid in shape (Fig. 8). It is interesting to note that Zacharias, in his observations on the division of the nucleus in living rhizoids of *Chara*, observed that in the process the nucleolus becomes irregular in shape, and undergoes amoeboid changes of form, and then disappears just before the formation of the chromosomes. The stage of this amoeboid condition of the nucleolus in *Chara* corresponds exactly with what is observed in stained specimens of *Phaseolus*. In *Phaseolus* the amoeboid condition of the nucleolus coincides exactly with the thickening of the nuclear threads connected with it, and an inspection of Figs. 14 to 16 indicates pretty clearly that as the nucleolus decreases in size the nuclear thread becomes more and more prominent, while still in definite continuity with the nucleolus, and staining reactions show that it becomes stained in a similar way.

While these changes are taking place, kinoplasmic fibres appear at the poles of the nucleus, elongated in the direction of the future spindle. The nucleus contracts, and the nuclear thread tends to be drawn more closely around the nucleolus (Fig. 10). The nuclear wall breaks down gradually, and the fibres penetrate the cavity of the nucleus (Figs. 11 and 12). This may take place before the formation of the chromosomes, and the spindle-fibres may come into contact with the unsegmented nuclear thread (Figs. 15 and 16).

The thread then becomes bent sharply at different points, the nucleolus still being in connexion with it (Fig. 16), and then breaks up into short rod-like chromosomes. Fig. 17 indicates a portion of a nucleus at this stage, in which can be seen the remnant of the nucleolus and some of the chromo-

¹ The reference to the Figure in Němec's paper, p. 316, appears not to be correct, it seems to be Fig. 5, not Fig. 42, to which his description refers.

somes just on point of separation; Figs. 13, 18, and 19 show various appearances of the nuclei at this stage, with an irregularly shaped indefinite nucleolar mass surrounded by chromosomes. The chromosomes become shorter and thicker, and arrange themselves to form the nuclear plate (Fig. 20). The remains of the nucleolus can still be seen, and in Fig. 20 there is still visible an indication of its connexion with the chromosomes in the faintly stained band between the drawn-out portion of the nucleolus-remnant and the chromosomes (cf. Fig. 16). It appears that the numerous connecting threads around the nucleolus gradually disappear, until only one is left, the nucleolus being drawn out at this point into a kind of tail (Figs. 16 and 20).

It is extremely difficult, however, to be certain of the exact sequence of events, as the observations have to be made entirely on stained specimens. In many cases the nucleolus appears as if it was becoming directly transformed into chromosomes (see Figs. 12 and 13), but this I think is due to a contraction of the nuclear network around the nucleolus just at this time.

Finally all connexion of the nucleolus with the chromosomes ceases, It is now much smaller in bulk, stains less intensely than before, and often exhibits a somewhat spongy texture, and at the same time begins to divide into two generally unequal portions (Fig. 21) which separate to opposite poles of the spindle-figure (Fig. 23). Rosen has already observed this phenomenon of nucleolar division in *Phaseolus*, and similar phenomena have been observed in some other plants. These nucleolar remnants are also surrounded by a clear space as in the resting nuclei, and in Fig. 22 is seen a case in which the single nucleolar remnant left at one of the poles of the spindle is surrounded by a clear space across which suspending fibres are seen.

At a later stage these nucleolar remnants entirely disappear, and coincident with this the spindle becomes more prominent: the fibres increase in number and stain more deeply, so that one might easily conclude that there was some connexion between the two, and that a portion of the nucleolus is concerned in the formation of the spindle.

RECONSTITUTION OF THE DAUGHTER-NUCLEI.

We have now to consider the changes which take place in the chromosomes during the reconstitution of the daughter-nuclei. These have an important bearing upon the question of the relation of nucleoli to chromosomes, for it is clear I think, from the observations about to be described, that the nucleoli in the daughter-nuclei definitely originate by the fusion of the chromosomes, first of all into a number of small nucleolar masses, connected together by a deeply stained network, and then by a further fusion into the large nucleoli found in the mature cells.

The equatorial plate first of all splits into two groups of daughter-chromosomes which separate to opposite poles of the spindle. As this takes place the chromosomes are distinctly seen as very short rods or granules (Figs. 25, 26). Whether they are actually separated from one another or remain more or less connected together by fine anastomosing threads I could not definitely determine. On arrival at the poles of the spindle they become aggregated together into a more or less homogeneous mass, in which the chromosomes can with difficulty be recognized (Fig. 27). At this stage the cell-plate appears across the equatorial region of the connecting fibres which are now very numerous. A nuclear membrane then appears, and the nucleus begins to open out or expand, exhibiting the chromosomes connected together by a deeply stained network (Fig. 28). The nucleus appears at this stage as if lodged in a cavity in the cytoplasm, the limiting layer of the nucleus or nuclear membrane apparently consisting of the peripheral layer of the nuclear network in close contact with the cytoplasmic layer¹. The cell-plate now gives place to the new cell-wall in the middle region of the figure, from which the connecting threads are fast disappearing, but at the periphery of the spindle the cell-plate formation is still going on, as indicated by the prominent and deeply stained connecting fibres. There is no indication whatever of any concentration of the spindle-threads to form nucleoli, as Němec states, although it is quite possible that a portion of them have been absorbed into the daughter-nuclei, and may enter into the constitution of the prominent network with which the chromosomes are connected.

The chromosomes now begin to fuse together into somewhat irregular masses, or in some cases into a thick, irregular band or thread, still connected by a well-defined and deeply stained network of threads (Fig. 29). The connecting fibres in the middle region of the cell have disappeared, but those at the periphery of the nucleus are still very prominent. Already there are indications that the nucleolar masses are the centres of radiation for suspending fibres. This becomes very clear at a later stage, as shown in Fig. 31, in which the nuclei contain two and four nucleolar masses respectively. Around each large mass and one of the smaller ones clear spaces are to be seen.

Fig. 30 shows a still further stage of chromosome fusion. In the upper nucleus there are now only two large nucleolar masses, and the way in which these have arisen by the fusion of smaller ones is shown in the lower of the two nuclei, in which four smaller nucleolar masses are to be seen fusing together in pairs. The nuclear network is still very prominent, and numerous granules of chromatin-like substance are visible on it. The

¹ Grégoire and Wygaerts, *Beihefte, Bot. Cent.*, 1903, p. 13, and Lawson, *Bot. Gaz.*, xxx, 1903, p. 305, also conclude, from their observations on various plants, that the nucleus is lodged in a cavity similar to a cell-vacuole.

cell-wall now extends nearly all across the cell. The formative fibres are completely separated from the nuclei, and appear free in the cytoplasm at the periphery of the cell, as shown in Figs. 30 and 31.

Fig. 31 shows a later stage of chromosome fusion. There are in each nucleus one large nucleolar mass, which has a form indicative of its production by the fusion of two smaller ones, and one or more smaller ones. In the upper nucleus one of the smaller nucleolar masses appears to be on the point of fusing with the larger. In both nuclei the large mass and one of the smaller ones is surrounded by a clear space. In all the cases of division figured, it is interesting to note that in each the two daughter-nuclei exhibit much the same conditions as regards fusion of the chromosomes, and are just in the same stage of development (Figs. 28-37).

Fig. 32 figures a case in which the transverse cell-wall is completely formed. In the daughter-nuclei are to be seen two large nucleolar masses in the same stage of fusion, and two smaller masses. The nuclear network is also clear, but with very few chromatin-granules.

In the next stage the fusion of the nucleolar masses is carried a step further (Fig. 33). In the lower nucleus there is one large nucleolus and near it are two smaller ones, of which one is just beginning to fuse with the large mass. The upper nucleus contains a single irregular nucleolus which has obviously just arisen by fusion with it of at least two smaller ones. I think we may consider, that the two prominent projections on it indicate the result of fusion of smaller masses with it.

It is not always the case that the result of the nucleolar fusion leaves the nucleus with one large and one or more smaller nucleolar masses. In Fig. 34 we have a case in which there are three about equal-sized masses of nucleolar substance which are just beginning to fuse together. In all these cases a delicate nuclear network is visible in contact with the fusing nucleolar masses, and in Fig. 35 is shown an interesting case of an irregular mass of nucleolar substance connected at one point in a very prominent manner with the linin-network (cf. Fig. 5).

When the nucleolar masses have become completely fused, the resulting nucleolus is at first a homogeneous irregular body of uneven outline (Figs. 33 and 35), but it gradually becomes more or less spherical (Fig. 36), and finally its homogeneity disappears, and it becomes vesicular (Fig. 37). As this proceeds the nuclear network becomes restricted to the peripheral part of the nucleus (Figs. 36 and 37), but the nucleolus remains connected to it, as already mentioned, by delicate connecting threads (Figs. 1-5).

During the time these changes are taking place, each nucleus gradually increases in size with the increase in size of the cell; the nucleolus also adds to its bulk, and goes on growing until, having reached a certain stage of development, the nucleus again begins to divide.

GENERAL CONSIDERATIONS.

It is not necessary here to enter into any detailed discussion as to the importance of the question of the function of the nucleolus. It will be sufficient to point out that the part ascribed to the nucleus, and especially the chromatin, as the bearer of the hereditary qualities in fertilization, renders necessary as precise a knowledge as possible of the chemical nature and function of each part of the nucleus before we can come to a definite conclusion that any one part or parts of it is more concerned in the process than another. The prominence of the chromosomes at certain stages during the division of the nucleus led to the enunciation of the doctrine that the hereditary qualities are transmitted by them; that they retain their individuality, more or less, in the resting nucleus; and that they must be regarded as individual units having an independent existence in the nucleus or cell.

The observations described in this paper show that we have in *Phaseolus* a phenomenon which, if it is found to be a widely spread one, must modify our conception of the significance of the chromosomes and nucleolus in heredity. The nucleolus is intimately bound up with the formation of the chromosomes, and Strasburger's contention that it is only concerned in spindle or kinoplasmic formation does not hold good, although it is not impossible that a portion of it—the plastin or pyrenin of Zacharias and Schwarz—may be used up in this way. There is no evidence either that the nucleolus originates from the spindle-fibres as stated by Němec; and Häcker's view that it is a product of excretion finds no support. A portion of it persists for a long time, even up to the stages of metaphase and anaphase, but it seems to disappear entirely within the region of the nuclear activity, that is in connexion with the chromosomes and spindle-fibres.

It seems clear also that the nucleolus does not originate *de novo* either from nuclear substance or from the cytoplasm. There is a definite continuity of nucleolar substance from mother-nucleus to daughter-nucleus through the chromosomes. How far this supports Zimmermann's conclusion, 'omnis nucleolus e nucleolo,' is perhaps difficult to determine. But if the nucleolus is simply a part of the nucleus in which nutritive substances are stored and perhaps partly elaborated, and not an independent nuclear organ, then, while there may be a definite nucleolar continuity, it seems to me that Zimmermann's conclusion simply becomes absorbed in the larger and more important—*omnis nucleus e nucleo*.

It is almost impossible to avoid coming to the conclusion that the nucleolus must be regarded simply as a part of the nuclear reticulum, in which chromatin-substance is stored for the use of the chromosomes during

division or other active condition of the nucleus. But it is not easy to arrive at any definite conclusions as to their chemical relations, or as to the exact rôle of the nucleolus in the metabolism of the nucleus. The increase in size of the nucleolus in the resting stage probably takes place at the expense of materials brought into the nucleus from the cytoplasm. In the transformation of these materials into chromatin there are, it seems to me, three alternatives as to the way in which they may be dealt with :—

(1) They may be taken up by the nucleolus directly from the cell-sap, and elaborated into chromatin, or (2) they may be first of all elaborated in the nuclear thread, and then passed on to be simply stored up in the nucleolus, or (3) they may be partly elaborated in the nuclear thread, and then passed on to be more completely elaborated in the nucleolus. Farmer's suggestion (*loc. cit.*, p. 512), 'that the nucleolus, though not in itself containing chromatin, is able to furnish at least one, and that probably the albuminous constituent of this substance,' would not explain the presence of the phosphorus-containing substance, which both Macallum and I find in such abundance in many vegetable-nucleoli.

Miss Huie¹ has made some interesting observations which bear upon this point and which seem to support the second, or possibly the third, alternative. In the gland-cells of *Drosera* during food-assimilation the nucleolus becomes smaller, whilst the chromosomes (nuclear network) become larger. The cytoplasm becomes impoverished and scanty in amount. Some time after feeding, the nucleus again becomes normal and the cytoplasm returns to its former condition. This indicates that the nucleus is the seat of metabolic activity, and that it is in the nuclear network, and not in the nucleolus, that the changes take place. But the diminution in size of the nucleolus, which is coincident with the increase in size of the nuclear network, seems to show that nucleolar substance is required before this metabolism can take place, or, in other words, that the activity of the nuclear thread is set up by the passage of nucleolar substance—chromatin or nuclein—into it, and that when the nucleus resumes its normal condition the nucleolus becomes restored to its original size by taking up the chromatin-material again from the nuclear thread². This is in harmony with Kossel's conclusion that the formation of new organic matter is dependent on the nucleus, and that nuclein (chromatin) plays a leading rôle in this process³.

It appears to be always the case, that when cells are in an active metabolic condition, the nuclear thread becomes prominent, whilst the

¹ Changes in the Cell-organs of *Drosera rotundifolia*, produced by feeding with Egg-albumen, Q. J. Mic. Soc., New Ser., xxxix, 1897, p. 387. Further Study of Cytological Changes produced in *Drosera*, Part II, Q. J. Mic. Soc., xlii, 1899, p. 203.

² Cf. Farmer, *Ann. Bot.*, ix, 1895, p. 495.

³ See Wilson, *The Cell*, &c., p. 340.

nucleolus becomes reduced in size or disappears, as in the guard-cells of stomata, in the gland-cells of *Chironomus*, and in the male sexual cells of plants and animals. In all such cases the nucleolar substance probably migrates into the nuclear thread, and remains there so long as the nucleus is in an active state, to be stored up in the nucleolus again when the activity ceases. Farmer and Williams¹ and Strasburger² have shown, for example, that the sperm-nucleus of *Fucus* is very rich in chromatin, and at the time of fusion with the ovum-nucleus exhibits a network-structure but no nucleolus. After fusion, a second large nucleolus appears in the ovum-nucleus, apparently derived from the chromatin of the sperm-nucleus. Here we might suppose that the male nucleus, on its entry into the quiescent female nucleus, loses its intense activity, and that the chromatin-substance therefore becomes accumulated in the form of a nucleolus.

So also Ikeda³ has shown, in his observations on the nutritive function of the antipodals in *Tricyrtis hirta*, that during their stage of metabolic activity the nucleolus decreases in size, whilst the original scanty chromatin-network shows an extraordinary increase of chromatin, which becomes variously aggregated within the nucleus.

It may be objected that chromatin-nucleoli, such as we have in *Phaseolus*, are only masses of chromatin-substance, and ought not to be confounded with those nucleoli which do not give a chromatin-reaction. There certainly appear to be two distinct types of nucleolus in some animal-cells, and it is possible that the same may exist in plant-cells also; but the evidence before us as to their chemical constitution, and staining, and other reactions, is not at present sufficient to differentiate them into two distinct categories⁴. We must rely mainly upon their morphological behaviour, and so far this has not produced much evidence of such a differentiation. As Montgomery suggests, it may be that all these bodies, which some observers are inclined to regard as fundamentally different structures, may be regarded as 'true nucleoli of a different chemical nature⁵.'

In conclusion, it appears to me, from a careful consideration of the facts as presented to us by various observers, that the following statements are probably justified as a summary of our present knowledge of plant-nucleoli:—

(1) That the rounded bodies present in nearly all plant-nuclei, which

¹ Fertilization of *Fucus*, Phil. Trans., 1898.

² Kernteilung und Befruchtung bei *Fucus*, Pringsh., Jahrb. f. wiss. Bot., xxx, 1897, p. 364.

³ Studies in the Physiological Functions of Antipodals and Related Phenomena of Fertilization in Liliaceae: (1) *Tricyrtis hirta*, Reprint from Bulletin, Coll. of Agriculture, Tokyo Imperial Univ., v, 1902, p. 41.

⁴ Cf. Fischer, Fixirung, Färbung und Bau des Protoplasmas, Jena, 1899, pp. 98–102, and Mann, Physiological Histology, Oxford, 1902.

⁵ Wood's Holl. Bio. Lectures, 1898.

differ among themselves as regards various stains and reagents, are all to be regarded as 'nucleoli.'

(2) That these nucleoli may be composed of plastin (or plastin-like substance) only, or of plastin combined with chromatin in varying quantities, and that this variation in composition partly accounts for the varied accounts which are given of their staining reactions, &c., in various plant-cells (cf. Montgomery).

(3) That in those cases where the chromatin-network is prominent and gives a strong reaction for chromatin, the nucleolus may either be absent or, if present, may give only a slight reaction for chromatin or none at all, and that where the chromatin-thread is not prominent the nucleolus is large and gives a strong reaction for chromatin.

(4) That the nucleolus simply forms a part of the nuclear network, in which chromatin or chromatin-substance may be stored, and possibly to some extent elaborated, and that it is not therefore an independent organ of the nucleus.

(5) That the nucleolus is concerned in the formation of the chromosomes, and possibly also in the production of the spindle, and that a portion of it may in some cases be extruded into the cytoplasm, and there disappear.

(6) That in the reconstruction of the daughter-nuclei the chromosomes unite together in a more or less irregular mass or thick thread, out of which is evolved the nucleolus and nuclear network, the major part of the chromatin passing ultimately into the nucleolus, except in cases where division again immediately takes place.

(7) That the vacuolar structure of nucleoli is general, and may indicate, either the separation or partial separation of the nucleolar substance into plastin and chromatin, or a greater accumulation of chromatin in its peripheral layer.

(8) That Zimmermann's conclusion, 'omnis nucleolus e nucleolo,' is not justified by the evidence before us, and that the nucleolus cannot, in the majority of cases at any rate, be regarded as an independent organ of the nucleus.

(9) If these conclusions are correct, it is obvious that the conception of the part played by the chromosomes in heredity will have to be modified, and, as Dixon has already suggested¹, the nucleolus, as well as the chromosomes, will have to be taken into account in any new hypothesis that may be put forward.

¹ Ann. Bot. xiii, 1899.

EXPLANATION OF FIGURES IN PLATE V.

Illustrating Mr. Harold Wager's paper on *Phaseolus*.

Fig. 1. Resting nucleus, showing the large vacuolar nucleolus and delicate threads suspending it to the peripheral network.

Fig. 2. Peripheral network seen from the surface of a resting nucleus. The linin-network with minute chromatin-granules is seen.

Figs. 3 and 4. Resting nuclei, with nucleoli containing a single vacuole of irregular outline, less deeply stained than the peripheral portion.

Fig. 5. A nucleus, showing the nucleolus with an interruption in the peripheral deeply stained portion at one point, giving it the appearance of a flask-like structure enclosing a less deeply stained substance. The suspending threads are more prominent and more numerous around this opening.

Fig. 6. Nucleolus of irregular shape with prominent suspending threads. The substance of the nucleolus appears as if drawn out into these threads.

Fig. 6a. Nucleolus, which had fallen out of a nucleus, showing threads drawn out of the substance of the nucleolus.

Fig. 7. Irregularly shaped nucleolus, showing its substance drawn out into the suspending threads.

Fig. 8. Later stage than Fig. 7. The nuclear membrane not clearly visible. The nucleolar threads are prominent.

Fig. 9. Surface-view of a nucleus at about the stage between Figs. 6 and 8. A portion of the peripheral network has become thicker and more prominent, with a delicate linin-network in the meshes of it. Compare with Fig. 2.

Fig. 10. A nucleus, showing the kinoplasmic filaments at the poles. The nuclear membrane is still visible.

Fig. 11. Nucleus, showing the nuclear membrane disappearing on one side, and the penetration of the kinoplasmic filaments into the nucleus.

Fig. 12. Slightly later stage than Fig. 11.

Fig. 13. The nuclear membrane has disappeared, and the bipolar spindle is now visible around the irregular mass of chromosomes and nucleolus.

Figs. 14-20. Show the formation of the chromosomes.

Fig. 14. A slightly later stage than Fig. 8. The connexion of the nucleolus with the nuclear network is well seen.

Fig. 15. Later stage. The filaments of the spindle are now in close contact with the nuclear network, which has not yet begun to break up into chromosomes.

Fig. 16. About the same stage as Fig. 15, but from a different point of view.

Fig. 17. A section taken through a nucleus at a later stage than Fig. 16, showing the segmentation of the nuclear thread into rod-like chromosomes.

Fig. 18. Shows the irregularly lobed nucleolus surrounded by chromosomes. About the same or slightly later stage than Fig. 19, but a larger nucleus. The connexion of the nucleolus with the chromosomes not visible.

Fig. 19. Section through a nucleus at about the same stage as Fig. 18, showing nucleolus and a few of the chromosomes.

Fig. 20. The grouping of the chromosomes in the equatorial plane. Equatorial plate. The spongy remnant of the nucleolus in the midst of them, less deeply stained, can be seen, with apparently some connexion still to the chromosomes.

Fig. 21. The less deeply stained remnant of the nucleolus shown dividing into two unequal parts, one of which will pass to each pole of the spindle. Compare Fig. 23.

Fig. 22. A nucleolar remnant at one pole only of the spindle. This is surrounded as in a resting nucleus by a clearer space across which suspending fibres are seen.

Fig. 23. Two unequal spherical nucleolar remnants approaching opposite poles of the spindle. Each is surrounded by a clear space, and stains much less deeply than the chromosomes.

Fig. 24. The nucleolar remnants have entirely disappeared; as they disappear it may be noted that a deeper staining and increase in number of the spindle-fibres takes place. An indication of this is shown in the Figures. Compare Figs. 21 and 24.

Fig. 25. Separation of the chromosomes. Anaphase.

Fig. 26. Later stage of anaphase.

Fig. 27. Fusion of chromosomes at ends of spindle to form the daughter-nuclei. Formation of cell-plate.

Fig. 28. Daughter-nuclei with chromosomes connected together by linin-network. The new cell-wall is beginning to form in the centre of the connecting fibres, which are now more numerous at the periphery where the formation of the cell-wall is still in progress, and will continue until it reaches the lateral walls of the cell.

Fig. 29. The chromosomes fuse together into larger masses, still connected by a linin-network. The central connecting fibre here entirely disappeared, leaving peripheral fibres only.

Fig. 30. Later stage of the chromosome-fusion. One daughter-nucleus contains two large masses only (nucleoli), the other four nucleolar masses, which are on the point of fusing together to form two. The cell-wall now extends nearly across the cell.

Fig. 31. Shows each daughter-nucleus at a later stage, with only one large nucleolar mass and one or more smaller ones. Each larger mass is surrounded by a clear space across which suspending fibres are visible as in a resting nucleus. In the nucleus in the upper part of the figure, two of the smaller nucleolar masses are in this clearer space, and one of them is on the point of fusing with the larger mass.

Fig. 32. Shows cell-division completed. The two daughter-nuclei contain fusing chromatin-masses and a linin-network.

Fig. 33. Later stage than Fig. 32. The fusion of chromatin-masses is nearly complete. In the upper daughter-nucleus there is a single irregular lobed mass. In the lower one a large spherical mass and two smaller ones, one of the latter is on the point of fusing with the large mass.

Fig. 34. Nucleus, showing the last stage in the fusion of chromatin-masses to form the nucleolus. The three masses have just begun to fuse together.

Fig. 35. The fusion is now complete, but the nucleolus has not yet rounded itself off.

Fig. 36. A still later stage, showing the nucleoli as more or less spherical homogeneous bodies.

Fig. 37. A still later stage, showing the vacuolization of the nucleoli. The daughter-nuclei are now in the resting stage.

WAGER.—ROOT-APEX OF PHASEOLUS.

Wager, Harold W. T. 1904. "The nucleolus and nuclear division in the root-apex of *Phaseolus*." *Annals of botany* 18, 29–55.

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