A DYNAMIC APPROACH TO TISSUE DIFFERENTIATION

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1. Early Views

THE problem of tissue differentiation has intrigued biologists of all ages. It would be refreshing, therefore, to commence an analysis with some of the earlier speculations. Spencer and Darwin postulated that the determiners of heredity circulated through the body. While the physiological units of Spencer were supposed to be alike, the gemmules of Darwin were as varied as the special cells they represented. Inheritance of acquired characters was conceded by both the investigators. According to Darwin environmental alterations get reflected in the gemmules produced by the tissues or organs. The method of transmission to the next generation was conceived to be by the collection of representatives of the different types of gemmules in the germ cells. A reversal of this process during ontogeny is a natural corollary. The products of division of embryonic cells are entered by gemmules representing the next stage of development which, by a process analogous to fertilization, determine their transformation into the type of body cell they represent in the adult organism (Herbert,[1] p. 54). In other words, during histogenesis, there is a "sorting out" of the hereditary particles. The views of these early biologists reveal the necessity felt by them to explain in a rational manner not only the origin of tissues but their integration in the scheme of organisation of an adult plant or animal.

Weismann who enunciated the germ plasm theory was critical of Darwin's views He argued that if the characteristics of cells are determined by gemmules, and the gemmules representing all the cells are present in the zygote, it is easy to explain the specific orientation of tissues and organs because the gemmules are available whenever and wherever they are wanted (Herbert,[1] p. 55). But then the necessity for an explanation of the integrated series of phenomena seen during development would still be there. He speculated that many somatic mitoses may not be equational "but in reality qualitative (erbunglich)" (Sharp,[2] p. 482). The mechanism for "sorting out" of the hereditary particles was conceived by Weismann to be in the nucleus itself. It would appear that this suggestion may turn out to be rather prophetic.

Embryonic development, Conklin[3] observes, "consists of differentiations built upon preceding differentiations" (p. 593) Since development is initiated by fertilisation, evidences for differentiation may be available at or immediately after fertilization. *Is the source of differentiation the nucleus, the cytoplasm or both* ? The discovery of the organ forming substances[3] in conjunction with the slow establishment of the dogma that all the cells of an organism have the same chromosome number[4] led to the view that it is the cytoplasm which plays a dominant role. It is this aspect which is emphasized in text-books. The argument is rather simple. Since histogenesis is not accompanied by a parallel differentiation of the gene complex, it has to be conceived that protoplasm with a given gene complex *is capable of* a wide variety of reactions in response to the changing local conditions during development. "In harmony with this interpretation is the fact that it is in the cytoplasm, rather than in the nucleus that most protoplasmic differentiation is manifested" (Sharp,[2] p 489)

The above conclusion is based on the following considerations. The chromosome number is constant in all cells of an organism and hence there can be no change in the gene complex during development The inference would be valid only if the facts are true A direct analysis of the inference is precluded owing to the following reasons (*i*) "The Mendelian theory has so far been concerned with heredity rather than development"[2] (p 489). (*ii*) Many of the morphological characters used in genetic analyses are products of irreversible differentiation. (*iii*) There is the whole world of embryonic development between the gene complex in the zygote and the determination and expression of the characters they represent in the adult[2] (p. 489). The concept that no change occurs in the gene complex during ontogeny is, therefore, not based on any direct evidence. It could perhaps have been justified, and that only in an indirect manner, if the chromosome number is constant in all cells of an organism. But is the chromosome number constant in all cells of an organism ?

2. Nuclear Phenomena Accompanying Tissue Differentiation

(*i*) *General.*—It has been recognised for some time now that the different tissues in the same

organism do not all have the same cytological make-up. "The statement made in most elementary accounts that all the nuclei of an organism contain the same number of chromosomes is only a first approximation to truth. Its frequent reiteration has, however, blinded many biologists to the fact, which has long been known (although its significance has only recently become apparent), that many of the differentiated cells of an adult organism are polyploid, that is to say, they contain 2, 4, 8, 16....times the diploid (2 N) number of chromosomes. This phenomenon is in general, only exhibited by the nuclei of cells which have differentiated so far that they have lost the power to divide by mitosis, unless reactivated to do so by some special treatment (*e.g.*, by growth-hormones in the case of plants)" (White,[5] p. 208).

(ii) Difference in the Number of Chromosomes between the Germ Line and Soma.—The earliest description of the difference in chromosome number between the germ track and soma is that of Boveri in *Ascaris*. The distinction starts even at the first cleavage, and becomes complete by the fourth. While in those cells destined to give rise to the germ track the chromosomes remain entire, in the others, they not only become subdivided into smaller pieces but their ends disintegrate in the cytoplasm[2] (p 489) The suggestion that the chromosomes of the germ line of *Ascaris* are really polycentric[6] would not explain the loss of the ends of the chromosomes during the crucial stages of differentiation into germ and soma. Do the changes indicate a primary alteration in chromosome balance between the germ and soma ?

(iii) Difference in the Number of Chromosomes between the Germ Line Cells, Mitotic Indifferent Cells of Different Sexes and Some Differentiated Tissues—A much more interesting example is *Sciara*.[7] There are generally 10 chromosomes in the spermatogonia and oogonia. The soma cells of the male showing mitosis have seven chromosomes while there are eight in the female. There is selective elimination of the paternal chromosomes excluding the X's and the 'limited' chromosomes during spermatogenesis. During the meiosis of the female, however, there is no irregularity. Immediately on fertilization, the zygote of *Sciara coprophila* contains one X derived from the egg pro-nucleus, two X's from the sperm and usually three L'S. This is said to be only a passing phase. "During the process of cleavage a number of the chromosomes are eliminated both from the somatic cells and from the germ line, so as to restore the chromosome sets characteristic of the adult tissues"[7] (p. 203).

Apart from the occurrence of the 'limited' chromosomes in the germ track alone and the difference in the number of X's observed in mitotically dividing male and female soma cells, further alterations are reported in the salivary cells. *In Sciara,* as in other insects, the salivary chromosomes are polytene. They however differ from those of *Drosophila* in the absence of a chromocentre[7] (p. 43). We thus see a variety of chromosome complexes in the same insect

It may be that *Ascaris* and *Sciara* are isolated examples where there is a difference in chromosome number in the embryo between even the mitotically dividing cells which eventually develop into the germ track and soma. Usually, the indifferent embryonic or meristematic cells and those of the germ track have an identical chromosome constitution. The major difference is between the meristematic or embryonic cells and those constituting the differentiated tissues. The nuclear changes observed when an indifferent embryonic cell starts on the road to differentiation have been described under a variety of terms. These diverse phenomena could be arranged in an ascending order of increasing complexity.

(*iv*) *Polysomaty.*—The occurrence of tetraploidy as a stage during differentiation has been reported in the case of *Allium*[8] and *Mimosa*.[9] One of the characteristic changes during differentiation is the lack of synchronization between chromosome reproduction and cytokinesis. While the case of *Allium* and *Mimosa* typifies skipping of a single cytoplasmic division, thus giving rise to the tetraploid condition a higher stage of chromosomal duplication is shown by *Spinacia*.[10,11] These *polysomatic* cells which exhibit an ascending series of chromosome complexity[11] are the result of the breakdown in synchronization between karyo- and cytokinesis at regular intervals. Double chromosomal reproduction in the interval between two cell divisions appears to be the mechanism responsible for the production of polysomatic cells. Polysomaty is characterised by (*a*) the occurrence of cells with multiples of the diploid number amongst meristematic cells, and (*b*) the ability of such cells to go through the same phases as the meristematic cells during division.

(*v*) *Endomitosis and Polyteny.*—Whereas there is only a lack of synchronization between chromosome reproduction and cell division in polysomaty, cytokinesis is absent in endomitotic cells. The connected series of events like chromosome reproduction as well as the separation of the replicas occur in the absence of any condensation of chromosomes and within an intact nucleus. Naturally, the chromosomes remain 'fuzzy' throughout the whole process[5] (p. 209).

Endomitosis is characterised by the individual chromosomes remaining discrete If the homologues fuse together, the products of their repeated division would give rise to the typical polytene chromosomes of the salivary gland cells of insects. An interesting case of the occurrence of both polyteny and polyploidy has been reported in *Lestodiplosus.*[5]

(*vi*) *Endopolyploidy.*—In many organisms, the occurrence of chromosomal replication within an intact nucleus has to be surmised from indirect evidence. In *Gerris* the heteropycnotic X chromosome has been utilized as a guide to judge the degree of polyploidy of the individual cells By counting the number of heteropycnotic X chromosomes in the resting nuclei Geitler concludes that the giant nuclei in the salivary glands may be 512- to 2048-ploid.[5]

Often, the variation in the number of heterochromatic bodies in the resting nuclei render accurate evaluation difficult.[12] Such cells which usually do not divide could be stimulated to do so by the use of plant hormones and thus reveal their chromosome complexity.[13]

The nuclei of the epithelial cells of the ileum of *Culex* larvæ do not show any heterochromatic bodies.[14,15] But from the somatic reduction divisions, that they undergo during metamorphosis, it has been estimated that they may be 32- or even 64-ploid.

Assessment of the degree of endopolyploidy based on the number of heterochromatic bodies or relative sizes of the nuclei can only be tentative. Though there is said to be a rhythmic increase in nuclear size accompanying chromosomal duplication in *Gerris*, instances are on record where, in spite of the hypertrophy of the nucleus, it was presumed to be diploid because it had only a single heteropycnotic X chromosome.[11] The premise on which such conclusions are drawn is that there is an equal duplication of all the chromosomes of the mitotic complement. Is there any justification for such a belief ?

(*vii*) *Differential Reproduction of Chromosomes.*—When a tissue becomes endopolyploid, it does not appear quite necessary that all the chromosomes of the mitotic complement should get duplicated to the same extent. In the nurse cells of the ovary of *Drosophila,* Schultz[16] reports—in an XXY form—that while the autosomes and the X chromosomes repeatedly reduplicate to give rise to the 512-ploid condition the Y chromosome is replicated only four times (p 36).

(*viii*) *Alteration in the Structure of Individual Chromosomes.*—*Ascaris* is not unique in that at a critical stage of differentiation loss of chromosome ends occurs in some cells. In the cortical parenchyma cells of *Allium* loss of telomeres have been reported.[17] As a consequence the free ends of the chromosomes that come into contact are said to have a tendency to fuse

The structure of the salivary chromosomes of insects has been under debate for the past two decades.[11] The technique which reveals the mid-prophase compound chromosomes of *Culex* uniformly stained shows the salivary chromosomes as consisting of alternating chromatic and achromatic discs. The removal of the achromatic regions from the salivary chromosomes, according to Berger[14] (p 230), would make them chromatic throughout as well as restore the normal length ratio expected between them and the metaphase chromosomes. Can we consider that there has been a differential reproduction of the different regions of the same chromosome ?

Compared to their length at mitosis, the heterochromatic regions are said to be much shorter in polytene chromosomes. Taking for example the X chromosome of *Drosophila melanogaster,* it is on record that while during mitosis about one-third of its length is heteropycnotic, the same region occupies less than one-tenth its length in the salivary nuclei[7] (p. 41)

There is no uniformity in the diameter of the euchromatin lying distributed along the length of the salivary chromosome. There are waist-like regions as well as localised swellings. The chromatic bands are said to be constituted by granules corresponding to the chromomeres of ordinary chromosomes. In favourable regions these granules could be counted and those of adjacent bands are connected by fine longitudinal threads. The number of chromomeres in adjacent bands may or may not be the same. Two threads converge on each

granule when there are only half the usual number of chromomeres in that particular band[7] (p. 38). During meiosis the chromomeres are known to differ in size.[6] But if there has been equal replication of all chromomeres, the number of granules in all bands of a salivary chromosome should be the same. Can we presume, therefore, that there has been differential reproduction of the granules constituting the different bands themselves?

(*ix*) *A Question of Terminology.*—The investigators who reported polysomaty,[11,14,15,18] polyteny, multiple chromosome complexes and endopolyploidy never made any serious attempts to relate these phenomena to tissue differentiation. As would be evident from the preceding pages it is possible to arrange the different cytological changes observed during histogenesis in an evolutionary scale. The most complex condition where there is not only an absence of cytokinesis but also visible evidence for the reproduction of most of the chromosomes could be derived from polysomaty with periodic lack of synchronization between chromosome reproduction and cytokinesis. Polysomaty, endomitosis, polyteny and endopolyploidy could, therefore, be considered as mere variations on a basic theme. The striking feature in all these phenomena is the replication of all or many of the chromosomes Histogenesis is a characteristic of higher organisms whether they be haploids, diploids or polyploids And, it is during histogenesis that one meets with a variety of cytological phenomena Clearly, they constitute a different order of changes unrelated to polysomy or polyploidy. It is to avoid confusion that Subramaniam[19,20] suggested the use of the general term *endopolyploidy* to refer specifically to the cytological phenomena observed during differentiation

3. Embryonic and Differentiated Cells

The analysis presented above reveals the desirability for a revival in a general way of the old Weismannian concept of *germ* and *soma*. This becomes imperative when it is realised that in *Sciara* there is a difference in the chromosome number between cells of the germ track and the indifferent cells of the soma, though both show mitosis. So long as there was an apparent justification for the belief that all cells in the same organism had the same chromosome number, there was no imperative necessity to differentiate between germ and soma. It is in the above context that one has to view the geneticist's limitation of the term "germ plasm" specifically to chromosomes[2] (p. 485). Now that ample proof regarding the invalidity of the law of constancy of chromosome number is available,[4,12] a revival of an analogous but fundamental distinction becomes necessary.

Admittedly while the instance of *Sciara* would suggest a rigid classification, it would be preferable to broaden it in view of the general identity in chromosome number between the indifferent cells of the germ track and the embryonic or meristematic cells. The disintegration of the primordial germ cells and the origin of the functional germ track from the indifferent cells of the early embryo are regular developmental phenomena in some insects[2] (p. 490). The number and behaviour of chromosomes are identical during *normal mitosis* whether it be in a cell of the embryonic tissue or the germ track. So long as they do not undergo any differentiation, mitotic cells are potentially immortal.

Lack of synchronization between chromosome reproduction and cytokinesis produces two distinct types of transformation. Functional germ cells *come into being as a result of chromosome reproduction being out of step with cytokinesis*[6] (p. 88). On the other hand, *suppression of cytokinesis to varying degrees*, during histogenesis, results in conditions described as polysomaty, endomitosis, polyteny and endopolyploidy.[21] We see thus that specialized cells whether they be the sperm or ova or those constituting the diverse tissues of an organism are *products of differentiation in opposite directions* They resemble one another in that they have only a limited span of life. It would be desirable, therefore, to classify cells into two broad categories, *viz.*, (*i*) EMBRYONIC, and (*ii*) DIFFERENTIATED. Tissues may contain embryonic cells for purposes of replacement of those lost due to senility and death

4. Polyploidy and Endopolyploidy

Once the necessity for such a primary distinction is grasped, it would be obvious that polyploidy and endopolyploidy are unrelated phenomena. It is rather interesting to recall that Winkler[4] attempted such a distinction as far back as 1916. "The constancy of chromosome number is safeguarded even when there is vegetative reproduction, since plants grow with their growing points which, by definition, are always embryonal.....We therefore come to the view that the regular occurrence of polyploid cells in the somatic tissues of higher plants by no means refutes the law of constancy of chromosome number but must be

expected in view of the importance of the chromosome number for cell size"[4] (p. 13).

Apparently Winkler was trying to emphasize the salient fact that separation of diploids from polyploids is based on investigations of chromosome number in embryonic cells. Embryonic cells in diploids as well as polyploids are capable of differentiation into germ cells or as components of various tissues. And it is during tissue differentiation that the cells become endopolyploid. There is thus no reason to consider that polyploidy and endopolyploidy are interchangeable phenomena Nor can it be assumed that viable polyploid types could be derived directly from endopolyploid cells.

The cytological events during histogenesis, therefore, can have a significance only to the origin of tissues and not, as in the case of polyploidy, to the hereditary make-up of the organism.

1. Herbert, S., *The First Principles of Heredity*, Adam and Charles Black, London, 1910.
2. Sharp, L. W., *Introduction to Cytology*, McGraw-Hill & Co., 1926.
3. Conklin, E. G., *General Cytology*, Ed. E. V. Cowdry. The Univ. of Chicago Press, 1925.
4. Huskins, C. L., *Int. Rev. Cytology, I.* Academic Press Inc., 1952, 9-26.
5. White, M. J. D., *Cytology and Cell Physiology*, Oxford Univ. Press, 1951, 183.
6. Darlington, C. D., *Recent Advances in Cytology*, 1932.
7. White, M. J. D., *Animal Cytology and Evolution*, Camb. Univ. Press, 1945.
8. Berger, C A. and Witkus, E. R., *Am. J. Bot.*, 1946, **33**, 785.
9. Witkus, E. R. and Berger, C A., *Bull. Torrey Bot. Club*, 1947, **74**, 279.
10. Berger, C. A , *Bot. Gaz.*, 1941, **102**, 759.
11. Lorz, A. P., *Bot. Rev.*, 1947, **13**, 597.
12. Huskins, C L., *Amer. Nat* , 1947. **81, 401.**
13. Huskins, C. L and Steinitz, L. M., *J. Hered.*, 1948, **39**, 67.
14. Berger, C A., *Carnegie Instn. Wash Publ Contr. to Embryology*, 1938, **167**, 211.
15. Grell, S. M., *Genetics*, 1946, **31**, 60.
16. Schultz, J., *Expl. Cell. Res. Suppl* , 1952, **2, 17.**
17. Levan, A. and Lotfy, T., *Hereditas*, 1949, **35, 337.**
18. D'Amato, F., *Caryologia*, 1952, **4**, 312.
19. Subramaniam, M. K., *Proc. Nat. Inst. Sci. India*, 1948, **14**, 325.
20 Thiagarajan, T. R. and Subramaniam, **M. K.**, *Arch. f. Mikrobiol.*, 1954, **20**, 183.
21. Needham, J, *Biochemistry and Morphogenesis*, Camb. Univ. Press, 1942.

FRESH SOURCES OF SELENIUM

THERE is no known deposit of selenium as such which is worth mining. The element occurs with sulphide ores, and most of it is obtained as a byproduct in the electrolytic refining of copper. The "anode slime" formed in the process contains a fairly high proportion of selenium.

The U.S.A. is the biggest producer of selenium, all of it from this process, but its supplies are still not enough for its own industry, and it has to import more of it. Most of Great Britain's supplies of selenium come from Canada, again from copper refining plant. There are small quantities of selenium on the market which come from Sweden and Japan, but these are high priced compared with the Canadian selenium

There is a possible source of selenium in Great Britain which is now being investigated by the Chemical Research Laboratory as a result of a survey of the selenium problem by the Intelligence Division of the Department. Iron sulphide, or pyrites, is used in Great Britain in the manufacture of sulphuric acid. Like copper sulphide, it contains selenium. Flash roasting of pyrites is one of the processes which is used to avoid using sulphur as a raw material. The process is fairly new, but its use is expanding and it may produce quantities of selenium which would be worth recovering The selenium is concentrated in the wastes, dusts and muds from the roasting plant. Little is yet known of the economics of recovery, but waste material from three plants have been examined at the C R L The materials from one plant contain sufficient selenium to justify the hope that recovery would be worthwhile. As in copper refining the problem is to develop a method which will not interfere with the primary object of the process and be cheap enough and simple enough to make selenium production pay

The potential yield from this source will run into tons, a valuable addition to present supplies One of the speculative things about recovery is that pyrites varies so much in its content of selenium. The C.R.L. investigation shows, however, that the possibilities of augmenting supplies of this extremely valuable element in this way are well worth serious consideration.