CELL DIFFERENTIATION

A Ciba Foundation Symposium

Edited by
A. V. S. DE REUCK
and
JULIE KNIGHT

J. & A. CHURCHILL LTD.
104 GLOUCESTER PLACE
LONDON, W.1 1967

CELL DIFFERENTIATION

CELL DIFFERENTIATION

A Ciba Foundation Symposium

Edited by
A. V. S. DE REUCK
and
JULIE KNIGHT

J. & A. CHURCHILL LTD.
104 GLOUCESTER PLACE
LONDON, W.1 1967

First published 1967

Containing 43 illustrations

Standard Book Number 7000 1338 5

Library of Congress Catalog Card No. 67–26433

Contents

Membership

Symposium on Cell Differentiation, held 31st January–2nd February, 1967

Sir Alexander Haddow (Chairman)	Chester Beatty Research Institute, London
E. J. Ambrose	Chester Beatty Research Institute, London
E. Bell	Dept. of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts
M. L. Birnstiel	Institute of Animal Genetics, Edinburgh
J. Brachet	Faculté des Sciences, Université Libre de Bruxelles, Belgium
A. S. G. Curtis	Dept. of Zoology, University College, London
W. A. Engelhardt	Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow
M. Feldman	Weizmann Institute of Science, Rehovoth, Israel
G. P. Georgiev	Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow
C. Grobstein	Dept. of Biology, University of California, San Diego, California
J. B. Gurdon	Dept. of Zoology, Oxford
K. S. Kirby	Chester Beatty Research Institute, Pollards Wood Research Station, Chalfont St. Giles
J. W. Lash	Dept. of Anatomy, University of Pennsylvania, Philadelphia, Pennsylvania
A. Monroy	Istituto di Anatomia Comparata, Università di Palermo, Sicily
J. Paul	Cancer Research Department, Royal Beatson Memorial Hospital, Glasgow
M. D. Rosenberg	College of Biological Sciences, University of Minnesota, St. Paul, Minnesota
G. V. Sherbet	Chester Beatty Research Institute, Pollards Wood Research Station, Chalfont St. Giles
R. Vendrely	Institut de Recherches Scientifiques sur le Cancer, Villejuif (Seine), France
C. H. Waddington	Institute of Animal Genetics, Edinburgh
P. A. Weiss	Rockefeller University, New York
M. E. Whisson	Chester Beatty Research Institute, London
Et. Wolff **Em. Wolff**	Laboratoire d'Embryologie Expérimentale, Collège de France, Nogent-sur-Marne, France
T. Yamada	Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

The Ciba Foundation

The Ciba Foundation was opened in 1949 to promote international co-operation in medical and chemical research among scientists from all parts of the world. Its house at 41 Portland Place, London, has become a meeting place well known to workers in many fields of science. Every year the Foundation organizes from six to ten three-day symposia and three or four one-day study groups, all of which are published in book form. Many other informal meetings also take place in the house, organized either by the Foundation or by other scientific groups needing a place to meet. In addition, bedrooms are available for visiting scientists, whether or not they are attending a meeting in the building.

The Ciba Foundation owes its existence to the generosity of CIBA Ltd, Basle, who, realizing the disruption of scientific communication caused by the war and by problems of distance, decided to set up a philanthropic institution whose aim would be to overcome such barriers. London was chosen as its site for reasons dictated by the special advantages of English charitable trust law, as well as those of language and geography.

The Foundation's many activities are controlled by a small group of distinguished trustees. Within the general framework of biological science, interpreted in its broadest sense, these activities are well summed up by the Ciba Foundation's motto, *Consocient Gentes*—let the nations come together.

Preface

We owe this meeting to the initiative of Sir Alexander Haddow and Professor C. H. Waddington, who suggested that one of the Ciba Foundation's small international symposia should be concerned with Cell Differentiation. The Foundation was more than happy to agree with this proposal, and it is a pleasure now to express our heartfelt thanks, not only to Sir Alexander and Professor Waddington, but also to Professor E. J. Ambrose and Professor Michael Feldman, for the care and time that each devoted to helping with the organization of the programme and to suggesting the participants.

The Foundation, the members of the symposium and also the readers of this book have further reason to be grateful to Sir Alexander Haddow, as chairman, for his skilful and gentle guidance of the discussions.

If more questions are raised than answered in this symposium, we feel that this may in itself be a fruitful contribution to progress in this vital field.

CHAIRMAN'S OPENING REMARKS

SIR ALEXANDER HADDOW

ALL assembled here are constantly preoccupied by what is still the great mystery of differentiation. In my case, and for several others here, there is a special applicability to the problem of cancer, where it is basic. In my own laboratory we have not been able to contribute directly to fundamental studies of differentiation until more recently, but I have been struck by the approach of Dr. R. J. Goldacre, over the past few years, in his construction of ingenious electronic models involving the passage of signals from one unit to another in symmetrical ways and eventually demonstrating the emergence of polarities and new kinds of symmetry and asymmetry. Models such as these may seem remote from cell differentiation, but they have caused us to think a great deal about interactions between cells and their relationship to the development of differentiated patterns. And of course from the beginning there has been clearly recognized in the cancer cell the presence of stages of de-differentiation. The relationship is not entirely simple, but perhaps near-perfect. We recognized structural de-differentiation; still later functional de-differentiation; and, most recently and in much greater chemical detail, biochemical loss and deletion. All this must ultimately involve studies of energy relations, and it may be justifiable to regard what we call the normal state and the malignant variant as constituting two separate energy levels, the transition from one to the other being facile and the reverse extremely difficult or thus far impossible.

When the German schools of pathology first examined the microscopy of the cancer cell they were immediately struck by the analogy with the embryonic state. Some have recently felt that in spite of the work of the intervening 140 years, we have overlooked this, and that now is the moment for further study of the analogy. There is a good deal of old biochemical work suggesting that in the embryonic cell many enzymic systems that are found later are ostensibly absent, but we realize that the precursors are present, and the old suggestion was of an orderly emergence of these regulatory systems, slowly bringing cells into various kinds of differentiation and relenting their speed of growth. In the last few years we have had much evidence to suggest that the cancer cell is similarly defective but permanently so, and it is possible that here is the key difference.

Furthermore, for many years we and others have compared the special biochemical properties of the cancer cell with what we called the corresponding normal cell. The great bulk of these studies utilized the corresponding normal *adult* cell, and we now begin to see how misleading this comparison can be. It is likely that the real comparison ought to be between the cancer cell and a normal cell growing equally rapidly—perhaps the embryonic cell. There is yet another point of view: what is the definition of normality? Some would claim that biologically speaking the cancer cell is normal and that abnormality resides in the adult cell, with its extraordinary built-in systems of regulation and control.

What we need most is a fresh study of the chemistry of differentiation towards much greater precision. There is an analogy here. My great predecessor at the Chester Beatty Institute, Sir Ernest Kennaway, who was responsible for establishing that the cyclic aromatic hydrocarbons are potent carcinogens, was responsible with his school for the isolation and recognition of benzpyrene in carcinogenic coal tar. I would be the last to diminish in any way this tremendous contribution. But I am struck by the fact that his work on the isolation of the hydrocarbons from pitch, which required an immense amount of starting material and took many years of labour and great ingenuity, could now, such is the advance of chemical technology, be carried out in a single afternoon. It may be that we are faced by something of a similar nature but immensely more complex and difficult, namely, the isolation and recognition of those substances in the embryonic cell and its environs which are responsible for the processes of differentiation. But immensely more difficult as that must be, advances in chemical technology still continue at an unbelievable pace and it could be that in the next decade or so these problems will become amenable to solution.

In conclusion, a feature of Ciba Foundation symposia is the emergence of new ideas in discussion, which is, I think, only possible in a small closed meeting of this type. One matter which concerns me a great deal at the moment is the need for more effective collaboration between those of us in different laboratories interested in this general field, not only in relation to the cancer problem. We all know how difficult it is in practice to establish such co-operation, but this is perhaps something to which we should give more thought, as a possible beneficent outcome of our present symposium.

GENERAL REVIEW OF THE NATURE OF DIFFERENTIATION

M. ABERCROMBIE

Department of Zoology, University College, London

THIS introductory paper is an attempt to consider some general ideas on the nature of differentiation. I shall not discuss everything that has been called differentiation, but one fairly well delimited category of cell change, namely that involved in the establishment of the different kinds of tissues in vertebrates. The framework of ideas considered is not original; it will be obvious that it is compounded of a great deal of Nanney (1958, 1960), Paul Weiss (1939, 1953) and Grobstein (1959, 1966), with contributions from many other authors, not all of whom are named.

I shall pay most attention to the finished products of the processes of differentiation, on the grounds that it is necessary to understand the nature of the differences between the tissues before analysis of their origin is effective; and I shall try to bring into relief one main point about these differences. The point is that the different tissue types are in a formal sense like different organisms in a genetically mixed population. The organisms can be treated as the phenotypic expressions of different genotypes. The argument is that the tissues are similarly to be treated as the phenotypic expressions, not of different genotypes, but of something closely analogous, which Nanney (1958) called epigenetic systems (he applied the term also to similar systems in micro-organisms).

In order to assess the argument in favour of this formal analogy, it is first necessary to set out the elementary steps on which the genotype–phenotype analysis of organisms is based. I shall set it out in four steps, as follows.

(1) *Multiplication.* Each organism may produce several offspring.

(2) *Inheritance.* In doing so, organisms show inheritance, each reproducing according to its own kind, and differently from other kinds.

(3) *Intrinsic differences.* The reason that the different kinds reproduce differently is not (or not only) because when they do so they are situated in different environments. When two different kinds are placed in a common environment they continue to reproduce differently. They are

therefore intrinsically distinct. This intrinsic distinction, in order to be handed on with multiplication from generation to generation in a common environment, must be self-reproduced.

(4) *Lack of continuity of phenotype.* What is transmitted from generation to generation with self-reproduction is not the whole structure of the organism, because the transmission can take place, without breaking the chain of heredity, through links of very different form. The sperm and egg with their associated developmental stages are the standard example of such a link, but more relevant to the analogy with tissue types is that one generation can, as a result of environmental influences, deviate from its parents, and yet (neglecting genetic segregation) the original character can be restored in the succeeding generation by return to the original environment. It is necessary therefore to say that the continuity is provided by an unaltered part of the organism, the genotype, which generates the phenotype, with the co-operation of environmental influences. The genotype must be self-reproducing. To suppose otherwise is to become lost in the infinite regress of explanation which the discovery of the self-reproduction of DNA finally disposed of.

These four steps in the argument must now be examined in detail in terms of the different tissues.

(1) *Multiplication.* Each cell can produce more cells. It is necessary to assert this point because it is so often said that differentiated cells do not divide. I shall not be able to list here the instances where they do divide during post-natal growth or reparative processes in vertebrates (see Abercrombie, 1957). There are many. It is undeniable, however, that the decline with age of the specific growth rate of tissues produces a (rather poor) negative correlation with the increase in differentiation. And there are some tissues, commonly continuously produced by stem cells, that do not show division (red blood cells as a class are not among them).

(2) *Inheritance.* When a given kind of differentiated cell multiplies within the organism (or in tissue culture) successive generations are alike if conditions remain steady. Some clear exceptions apart, this proposition would probably be generally acceptable, and does not need documentation.

(3) *Intrinsic differences.* Given two different reproducing tissues in an organism, does the propagated difference depend on the influence of their different surroundings or is it intrinsic to the tissues, or is it a mixture of both? The procedure for answering this question is simple in principle: the tissues have to be exchanged by mutual transplantation, and the products of their growth in their new situations examined. In seeking to

establish the analogy with heredity in whole organisms, we can make do with what is often a technically simpler operation. We do not need to ask the specific question about a given difference between two tissues. We can ask whether there is *any* detectable intrinsic difference which is propagated and which distinguishes the two types (that is to say, is not found *within* either type). This is tested, as with whole organisms, by placing the tissues in any common environment (Weiss, 1953) and observing their ability to stay different during multiplication. (The exchange experiment also makes use of common environment, but there is a bonus of information because the two common environments tested are those belonging to the particular difference under analysis.) If in the common environment the two tissues, without necessarily maintaining the characteristics they have *in situ*, yet remain different during their growth, there is an intrinsic difference. If, on the other hand, they become indistinguishable during their growth, it is not possible to regard the converse as demonstrated, that they have no intrinsic difference: the environment chosen may be one that does not permit the intrinsic difference to manifest itself.

The answer experimentally obtained is perfectly clear, that different tissues remain different during their multiplication in a common environment (as for instance in tissue culture or in transplantation), and that these differences are not found within the same tissue. But these transplantations or explantations are as a rule of populations of cells. Do *individual* cells of different tissues remain distinct in a common environment? The evidence on this is far from satisfactory, as Grobstein (1959) has so cogently pointed out. Individual cells of different tissue types have often been put into a common environment in culture, during cloning experiments. These (for example, the comparison of fibroblasts and contractile cells of skeletal muscle by Konigsberg, 1963) demonstrate differences intrinsic to the isolated cells which affect their replication. Unfortunately they show only that the intrinsic differences suffice for one cell division. As soon as a division has occurred, each cell type is in a different environment—one that includes another member of its own type. Thenceforward each cell may fail to transmit any inheritance, but may be reinstructed by its neighbour. On a very strict definition of "intrinsic" one cell division in a common environment should be enough to demonstrate a replicable intrinsic difference. Yet this would be unrealistic. We are dealing with asexual reproduction, in which the daughter cells between them divide out most of the constituents of the parent cell. For multiple components, failure of replication would be manifested not by a sudden change but by a diluting out. It would be a useful experiment if someone would undertake the repeated

removal of one product of each of several successive divisions, after cloning various tissue types in various common environments.

It seems to be generally felt, however, in spite of the absence of the convincing evidence that Grobstein rightly demands, that the marked ability of vertebrate cells to maintain a replicated difference when populations of them are exposed to a range of common environments, *in vivo* and *in vitro*, justifies the hypothesis that individual cells carry intrinsic replicated differences independently of their neighbours. Whether or not this turns out to be wholly or partly true, the analogy with organisms can still be maintained; it is merely a question of the size of the units required to carry an intrinsic difference—whether a cell or a group of cells is to be compared with an organism.

(4) *Lack of continuity of phenotype.* The final step is to examine the parallel with the condition in organisms, in which it is clear that something that has stable properties is handed on from parent to offspring through stages of very different appearance, thus allowing a distinction to be made between genotype and phenotype. Cells in their reproduction do not go through a deviation of phenotype as marked as that of the egg stage in organisms. They may however change considerably during division. A Schwann cell of a myelinated fibre loses its myelin before it divides, for instance. More important is that cells can change their appearance very considerably as a result of exposure to a new environment, and change back again when the original environment is restored. This is precisely the category of change that Weiss (1939) has called modulation. It has recently been well exemplified, in a situation involving replication, by Coon and Cahn (1966). They have investigated a fraction of embryo extract which reversibly suppresses aspects of the specific synthesis of cartilage cells and of pigmented epithelium of the eye.

It seems evident therefore that in the heredity of some tissue types, something is handed on that is not co-extensive with the cell phenotype, but takes part, with the cell or tissue environment, in generating the phenotype. The latter may change reversibly from generation to generation as conditions change (modulation), while what is handed on remains stable. The genotype of organisms has therefore a parallel in differentiated cells, and this is what Nanney (1958) called the epigenetic system. As with the genotype, so with the epigenetic system we have to suppose that it is self-reproducing, or expose ourselves to an infinite regress of information transfer. Naturally, the epigenetic system itself is not to be thought of as completely stable. It can be assumed to undergo changes—transformations —under certain circumstances, and such changes, if tested by a common-

environment experiment, would prove to be intrinsic replicative changes. It cannot, however, be said that the metaplasias have yet been adequately tested in this way. Perhaps the most interesting changes from this point of view are those associated with malignancy.

There have been many discussions of the stability of cell phenotypes in varying conditions, which have seemed to imply that an unstable phenotype is incompatible with cell heredity. It will be evident from the argument I have been developing that a dependence of phenotype on environment is far from incompatible with cell heredity, as Weiss (1953) pointed out. If two cell lineages are different from each other in a common environment, we can argue that they carry different epigenetic systems. It does not matter if one or both change when put into a different common environment. But all we have done by this experiment is to establish the *existence* of epigenetic systems. We may be able to adduce evidence that these differences of epigenetic system are correlated with tissue type; but we cannot go further and argue that the differences which the tissues show *in situ*, the differences which in fact lead us to categorize them as belonging to different tissues, are due to these epigenetic systems. That could be further analysed by an exchange experiment, if such is technically possible. But it can be demonstrated that a difference of epigenetic system is implicated in the actual tissue difference if, when put in a common environment such as tissue culture, the tissue types maintain the phenotype that they manifest in the organism. In this sense stability of phenotype, though it is not essential for the demonstration of cell heredity, is a valuable asset in analysing the actual differences in an organism. Stability of phenotype is of course also an important subject in its own right (Waddington, 1957).

The application to tissues of an analogue of the analysis of whole organisms in terms of genotype and phenotype is not fanciful, since the experimental procedures required are in principle clear, though they cannot be said to have been systematically applied. There is furthermore no question that in the development of the genetics of whole organisms the distinction of genotype from phenotype was of the greatest importance. Can one make any such claim for the analogous distinction in the case of tissues? It has hardly attracted much attention from developmental biologists. In organisms the importance came from the fact that the genotype proved to be essentially a simple, clearly defined mechanism. It is therefore necessary to consider the likely mechanism of the epigenetic system.

Opinion is fairly generally in favour of the view that the base sequences of DNA do not distinguish the different tissue types, but that differential repression or release of the activities of the DNA, probably at transcription

level, does distinguish them. It is not possible to rule out the various entirely extra-genic systems that have been suggested, either of a structural kind such as Sonneborn's (1963) cortical inheritance in *Paramecium*, and the hypothetical plasmagenes, or some less localized kind of self-reproducing cycle. But it seems likely that for tissue differences in vertebrates these will, if they exist, prove to be only occasional adjuncts.

On the other hand, various temporary changes in the synthetic activities of cells, such as circadian and other intermittent fluctuations, and the modulations, for example hormonally induced, that we distinguish from changes in the epigenotype, may well also involve changes in gene activities. The essential difference between the systems that produce modulations and those that produce differentiations lies in the self-reproduction of the latter. The repression or activation of particular parts of the genotype is handed on. There need be no mystery about this self-reproduction. Various simple mechanisms have been suggested, such as those of Jacob and Monod (1963). If activators or repressors of gene function are involved, it is required that they should be produced in sufficient amounts to inhibit the new set of genes after its duplication, and that they should be divided between the daughter cells. There must also, however, be an arrangement to ensure that the presence of these regulator substances controls their own production in the required quantities. This can be visualized as a cycle, at least in so far as products of genome activity are directly or indirectly feeding back to the synthetic apparatus to engender the synthesis of more of these products, as well as the synthesis of the components responsible for the characteristic phenotype.

These self-reproducing cycles may be intranuclear, but nuclear transplantation (see Gurdon, 1963), as far as it has gone, suggests that they involve the cytoplasm too. These transplantations are experiments of the common-environment type, nuclei from different tissues being put into egg cytoplasm. So far they have failed to lead to clearly different development in this environment. This cannot be interpreted as demonstrating that the nuclei are intrinsically the same, but it could well mean that the nuclear part of the cycles concerned in differentiation is overwhelmed by the cytoplasmic part in the egg. But as argued by Grobstein, and already discussed, the cycles may be larger still, embracing a population of cells. In this case the individual cells will be manifesting a social heredity, not an intrinsic one.

The epigenetic system, according to views at present favoured, consists of a set of these regulatory agents, geared into the genome so as to reproduce themselves. Such a system may be, and apparently often is, thought too

simple, or too vague in content, or too potentially heterogeneous, to be dignified by a name, or to be ranked with something as profound and precise as the genome.

In these circumstances one should perhaps mention a different kind of argument for calling attention to epigenetic systems in differentiation. Developmental biologists may need no reminding that systems with these general properties exist, and will, in one form or several, play a part in a theory of differentiation. But one has the impression that for many biochemists, oncologists and virologists, who find cell transformations relevant to their interests, cell heredity means the base sequence of nucleic acids. Perhaps there would be a useful liberation of hypothesis if developmental biologists emphasized more the existence of epigenetic systems in vertebrates.

This raises the difficult question of terminology. "Epigenetic system" of Nanney should perhaps be considered to be pre-empted as a consequence of the long-standing use by Waddington and others of "epigenetic" to mean "developmental". The word "determined" of classical embryology is obviously related, but it was little concerned with replication, and is still identified in many people's minds with the idea of an unchangeable phenotype, challenged by Harrison (1933). Pursuing the form of the argument I have here developed, "epigenotype" would be a natural parallel to "genotype" and might be used for the set of self-reproducing regulatory mechanisms that characterizes each of the different tissue types of an organism. "Epigenotype" has been used by Waddington (1939) in a different sense, but infrequently. Any term will probably have only a temporary function and will soon be superseded by actual analysis of the mechanisms involved.

I have not so far discussed the process of differentiation, but only its results. Before these results are evident—that is, before the syntheses specific to the tissue type have come fully into play—there is in the vertebrate embryo a prolonged period of development during which the epigenetic systems (epigenotypes) are being established.

Earlier transplantation experiments of the Spemann era have shown that during this period there is a step-wise "restriction of potencies", as it was called, meaning that the range of variety of the differentiations that can readily be obtained from a given presumptive tissue by transplanting it within the embryo becomes progressively reduced. The restriction takes place in a characteristic way, which can be schematized as an arborizing temporal pattern, the terminal twigs being the different cell types that will

ultimately appear. The tissue proceeds from trunk to twig; and as it passes each bifurcation it loses the ability (insofar as this is tested by transplantation within the embryo) to form those tissues to which the rejected branch leads. The sub-divided classification of tissue types to which the arborizing pattern of development gives rise is correlated with the spatial lay-out of presumptive areas in the early embryo.

The experiments on which our picture of the course of the differentiation process is based were made with the relatively large blocks of embryonic tissue which are necessary in transplantation work. Within and between the cell populations transplanted, extensive interactions occur, which determine the progressive changes. Some of the more obvious have been analysed as successive inductions. Little, however, is known about the changes in individual cells during this period. At least one conclusion bearing on the discussion of epigenetic systems is nevertheless suggested by the earlier experimental work: the establishment in the embryo of an epigenotype responsible for differentiation into tissues is not the clamping at one stroke of a set of regulatory mechanisms on the genome. In terms of the theory at present favoured, an epigenetic system seems to be constructed by the successive removal of blocks of the genotype from easy access by whatever it is that produces the final synthetic activity.

During this process of establishment of the epigenotypes, the parallel with the genotype–phenotype analysis of organisms may not be useful. It is not clear whether there are successive transformations, each step producing a modified epigenotype which is stably self-reproducing in the absence of further inductive influences. Experimental choriocarcinoma suggests that such stable intermediates in the process of establishing the mature epigenotypes are a possibility (see Stevens, 1967). But insofar as the regulatory mechanisms during this process are not obtainable in a stable self-reproducing form, the notion of an epigenotype cannot be usefully employed.

The long period of establishment is accompanied by changes in the characters of the cells, particularly in those properties, such as contact relations between cells, that affect the profound morphogenesis in progress at this time. The history of the presumptive axial cartilage cell in a chick or mammal embryo, for instance, seems to show an alternation between epithelial properties (epiblast stage, somite stage) and mesenchymal properties (primitive streak stage, dispersing sclerotome stage), which one would guess represents an elaborate series of changes in contact properties, all occurring before cartilage differentiation is apparent. Presumably these transient states include also the output of and receptivity to the signals between cells that are guiding development.

At the end of the period of establishment of the epigenotype, the selected constellation of genes for the particular tissue type comes into full synthetic activity, and visible differentiation begins. Subsequent changes in tissues are reasonably assumed to be, for the most part, modulations. At least one class of changes, however, involves a clear inherited transformation: these are the changes concerned in malignancy and its progression. They do not as a rule include any change in tissue type, though what can be presumed to be gene activity inappropriate to the tissue of origin has been detected, as in the production of anti-diuretic hormone or ACTH by lung carcinomas (Meador *et al.*, 1962; Amatruda *et al.*, 1963). They do involve heritable changes in response to the controls of movement and mitosis. Clearly the possibility that these are alterations in systems of the same general sort as the epigenotypes I have discussed, conceivably comparable to the changes in contact relations that occur during the establishment of epigenotypes, needs at least to be borne in mind (see Harris, 1964).

Jacob and Monod (1963) remarked that differentiation is present when cells with the same genome synthesize different proteins. In this sense I have been considering only a very restricted part of the full range of phenomena. It may well be misleading to generalize from vertebrates to other multicellular organisms even about the differentiation of tissue types. There is a good deal of relevant information from the cell culture of higher plants which suggests that heritable differences may not characterize at any rate many of their tissues. Differentiation here, as perhaps in other groups not yet studied, may depend on modulation, associated with a substantial absence of mitosis in the differentiated cell.

SUMMARY

A comparison of the inheritance of differences between tissue types in a vertebrate with the inheritance of differences between organisms suggests that the distinction between genotype and phenotype made in the latter has a close formal parallel in the former. This notion that tissue cells possess self-reproducing systems analogous to the genotype has been put forward especially by Nanney and Weiss. Nanney called them "epigenetic systems". It is suggested that, if a name is required, "epigenotype" might be useful for the set of such systems characterizing a tissue type.

It has, however, been rightly pointed out by Grobstein that the experimental evidence is inadequate to localize these systems within the individual cell. The possibility is still open that the epigenetic systems, or some of them, are population effects, and that the formal analogy should be between cell population and organism.

As various authors have shown, there is no difficulty in constructing plausible models of such self-reproducing systems. Their actual establishment during embryonic development is a surprisingly complex and lengthy procedure.

REFERENCES

ABERCROMBIE, M. (1957). *Symp. Soc. exp. Biol.*, **11**, 235–254.
AMATRUDA, T. T., MULROW, P. J., GALLAGHER, J. C., and SAWYER, W. H. (1963). *New Engl. J. Med.*, **269**, 544–549.
COON, H. G., and CAHN, R. D. (1966). *Science*, **153**, 1116–1119.
GROBSTEIN, C. (1959). In *The Cell*, vol. 1, pp. 437–496, ed. Brachet, J., and Mirsky, A. E. New York: Academic Press.
GROBSTEIN, C. (1966). *Am. Zool.*, **6**, 89–95.
GURDON, J. B. (1963). *Q. Rev. Biol.*, **38**, 54–78.
HARRIS, M. (1964). *Cell Culture and Somatic Variation*. New York: Holt, Rinehart and Winston.
HARRISON, R. G. (1933). *Am. Nat.*, **67**, 306–317.
JACOB, F., and MONOD, J. (1963). In *Cytodifferentiation and Macromolecular Synthesis*, pp. 30–64, ed. Locke, M. New York: Academic Press.
KONIGSBERG, I. R. (1963). *Science*, **140**, 1273–1284.
MEADOR, C. K., LIDDLE, G. W., ISLAND, D. P., NICHOLSON, W. E., LUCAS, C., NUCKTON, J. G., and LUETSCHER, J. A. (1962). *J. clin. Endocr. Metab.*, **22**, 693–703.
NANNEY, D. L. (1958). *Proc. natn. Acad. Sci. U.S.A.*, **44**, 712–717.
NANNEY, D. L. (1960). *Am. Nat.*, **94**, 167–179.
SONNEBORN, T. M. (1963). In *The Nature of Biological Diversity*, pp. 165–221, ed. Allen, J. M. New York: McGraw-Hill.
STEVENS, L. C. (1967). *Adv. Morphogen.* **6**, in press.
WADDINGTON, C. H. (1939). *Introduction to Modern Genetics*. London: Allen and Unwin.
WADDINGTON, C. H. (1957). *The Strategy of the Genes*. London: Allen and Unwin.
WEISS, P. (1939). *Principles of Development*. New York: Henry Holt.
WEISS, P. (1953). *J. Embryol. exp. Morph.*, **1**, 181–211.

DISCUSSION

Waddington: When I first used the term "epigenotype", it was in a different sense from yours, as a general word for the epigenetic characteristics of the organism as a whole, namely its particular system of developmental interactions, whether it has organizers or gradients or some other system. The term is not much needed today in that sense and I am perfectly willing to give it up to somebody else! On the other hand, I am not really convinced by Nanney's use of it (Nanney, D. L. [1960]. *Am. Nat.*, **94**, 167–179). This was, I think, derived from M. Delbrück's idea ([1949]. *Unités biologiques douées de continuité génétique*, p. 33. Paris: Centre National de la Recherche Scientifique) that differentiation depends on cells being switched between alternative steady states. These ideas were developed in relation to the appearance of hereditarily stable variant clones during vegetative reproduction in protozoa such as *Paramecium* and *Tetrahymena*.

In my opinion, this is only a degenerate case of the switching between alternative pathways of change, which I have been discussing since at least 1939 (Waddington, C. H. [1939]. *Introduction to Modern Genetics*, p. 182 *et seq.* London: Allen and Unwin).

It is clear that differentiation is a matter of cell heredity, but many quite different things can be inherited, and when one considers one cell lineage during its development, it is clear that what is inherited (its "epigenotype") changes considerably. Thus, cells can inherit the state of competence, in which they are ready to be determined in some way. Or they can inherit "being determined"; the fashionable case is Hadorn's work on the imaginal buds of *Drosophila* (Hadorn, E. [1965]. *Brookhaven Symp. Biol.*, **18**, 148–161), but there are many other cases in tissue culture situations. Finally, the cells are activated, and produce pigment or whatever their final product is, and they can potentially go on reproducing in this state. In that case one has to say that the epigenotype of the same cell strain has passed through three difference stages, each of which is reproducible. This may be a good thing to say; certainly there is something inherited, and possibly we need a name for it, but if one uses only one name, it implies that it is always the same thing, which it probably is not.

Abercrombie: This is the point where my analogy between tissues and whole organisms breaks down. During the development of an epigenotype one cannot talk about inheritance in the kind of way I have been doing, because one does not know during these stages whether there is any inherited transformation. I would prefer not to press the analogy there, but to say that it applies to the differentiated state which is finally reached.

Weiss: I see some advantage in the term "epigenotype" as an important change of focus in the present situation, in which many people entering our field are unfamiliar with the actual process of "strain differentiation", hence are liable to be confused by the innumerable connotations attached to the term "differentiation". This confusion has reached the point where the mere labelling of the phenomenon of strain differentiation, in contradistinction to morphogenesis and cytodifferentiation, or whatever you will, is an important step. Regardless of what name one uses, the distinction of phenomena that are not cytodifferentiation—not simply the partial evoking or unblocking of the cellular genome as a result of the local circumstances to which it is subjected, implying reversibility—is an important achievement. Many people erroneously imagine that they study differentiation when they study the transformation of a myoblast into a muscle cell or the development of pigment in a melanoblast, which is already single-tracked and can never do anything except either produce melanin or not produce it; they fail to distinguish this from strain differentiation, where something is passed on to the cell progeny which breeds true throughout subsequent cell generations, even in changed and indifferent environments. Whether the true-breeding modifications find the same kind of expression that we expect from them in the natural environment, is a totally different problem. The basic point

that Professor Abercrombie has expressed so well is the preservation of *differentials* between strains of common genome, but different ontogenetic history, in a common environment, and not whether either of the differentiated lines bears any recognizable relation to its original form in the organism. Failure to take this fact into proper account has misled much good experimental work.

Abercrombie: As I said, I obtained these ideas from Paul Weiss anyway!

Lash: I don't want to labour this point, but if Professor Abercrombie means by "epigenotype" simply the genotypic expression which is translated into phenotypic expression, I see no advantage in using the term, particularly as it may be misleading for people not working in the field. As Professor Weiss mentioned, differentiation is not the transformation of the predetermined cell; differentiation is what determines or controls the genotypic expression of a complex of cells that can then be translated into a phenotypic expression.

Weiss: It is essentially the breaking up of one cell type and its offspring into different kinds, according to their acquired type specificities, that breed true—the differentiation of cell lines.

Abercrombie: I do not disagree with this, but I am using the word "epigenotype" for these controls, if you like to translate it this way, and I am saying that the whole system of these controls is inherited.

Waddington: How does this differ from the old ideas of determination? It seems to me that we already have a terminology for talking about the processes of change and of radical branching that occur within a cell lineage. Professor Abercrombie wants to use the word "epigenotype" as a general term for everything that this branching affects, which is perfectly valid, but it does not add much to the picture we had before, apart from an extra portmanteau term for all the factors which bring about the division of a cell lineage into two types of cell.

Feldman: I want to try to be a little more specific with regard to some of the processes that have been mentioned. I was glad that Professor Abercrombie referred to Sonneborn's observation of the inheritance of cell surface properties in *Paramecium*, which appear not to be determined by the direct control of the genes. Sonneborn has demonstrated that one strain of *Paramecium* can be "grafted" with a piece of cell membrane of a different strain (Beisson, J., and Sonneborn, T. M. [1965]. *Proc. natn. Acad. Sci. U.S.A.*, **53**, 275). When the "recipient" then replicates exponentially, the progeny of the grafted ciliate all manifest an area carrying the donor's marker. How then do the donor properties replicate themselves, if the graft did not contain, as is assumed, donor DNA or RNA templates?

In looking for models which may explain this type of "non-genetic" inheritance, we should consider some aspects of polysaccharide synthesis. It was demonstrated that *in vitro* synthesis of specific polysaccharides from monosaccharides can be obtained, provided that in addition to the monosaccharides, enzymes and energy sources, there are specific polysaccharide molecules in the

system. These function as a primer for further synthesis of the particular polysaccharide. This may provide a model for the phenomenon in *Paramecium*, assuming that the "graft" provides the primer, and the recipient, the enzymes, building blocks, and so on. In the absence of such primer, the specific surface structures would not have been formed. It seems to me that many other cell properties associated with surface structures may be determined by primer action, rather than by the more "conventional" template activity. For example, this may be the basis of the production of tumour-specific antigens, and of tumours induced by chemical carcinogens. We have demonstrated in mice that if the same carcinogen is applied to two symmetrical sites on an animal two tumours develop. These two tumours, originated from the same genetic background, possess *different* tumour-specific antigens (Globerson, A., and Feldman, M. [1964]. *J. natn. Cancer Inst.*, **32**, 1229). In view of the heterogeneity of the antigens of tumours produced by the same carcinogen acting on the same genetic background, and since these are cell-surface antigens, it appears to me that the antigenic changes may be derived from a direct interaction between the carcinogen and membrane components of the cell surface. Once such a change, manifested in a new antigenic determinant, has been produced, all cell progeny of the originally "transformed" cell will possess the antigen on the basis of a "primer" function of the originally "changed" structure.

I want now to direct a question to Professor Waddington on another phenomenon that Professor Abercrombie brought up. This is the demonstration of Cahn (Cahn, R. D., and Cahn, M. B. [1966]. *Proc. natn. Acad. Sci. U.S.A.*, **55**, 106) regarding the differentiation of replicating pigment epithelial cells, and of Coon regarding that of replicating chondroblasts (Coon, H. G. [1966]. *Proc. natn. Acad. Sci. U.S.A.*, **55**, 66), According to Cahn, pigment epithelial cells can replicate exponentially *in vitro* and manifest melanin formation if cultured in a medium containing the low molecular weight components of embryo extract. If now the cells are transferred to a culture medium containing the high molecular weight components of the embryo extract, they continue to replicate, without forming melanin. They can now replicate for many generations without synthesizing melanin. If, however, they are transferred to the first medium again, they again manifest melanin synthesis. A similar observation was made on chondroblasts and cartilage formation. These experiments indicate that a state of determination is replicable. What then is the state of the genes associated with the control of the determination or "commitment" to a certain direction of differentiation?

Waddington: Here there is being replicated some condition of the genome in which only certain genes can be activated, namely the genes that form pigment. Whether they *are* activated or not depends on something else. In an exactly similar way, except that it is not easily reversible, the imaginal bud of the wing of *Drosophila* can replicate many times and yet it does not form wing cells until the pupation hormone is given (Hadorn, E. [1965]. *Loc. cit.*). In the pigment cell

some condition of the particular DNA concerned with pigment formation must be replicating, and this is where one comes down to the molecular level. Let us suppose that the DNA concerned has some special type of histone or other chromosomal protein on it. Not only must the DNA replicate but so must the particular protein associated with it, and we have very little chemical evidence of how this is done. But although the DNA may replicate in this condition, it is not necessarily the condition in which it is active. People talk as if the essential step of differentiation is gene activation, but this is only the *last* step in the process. There can be an important replicating process when the genes are not active but are prepared to be active, and that is the more essential step to understand.

Lash: I have a partial answer to Professor Feldman's question. When chondrocytes are proliferating under conditions in which there is no phenotypic expression—no accumulation of cartilage matrix—they seem to perpetuate the genotypic expression of chondrocytes (Coon, H. G. [1966]. *Proc. natn. Acad. Sci. U.S.A.*, **55**, 66–73). That is to say, the metabolic pathways found in cartilage cells are present in these cells even though they do not accumulate polysaccharides unless grown in a permissive medium (G. Marzullo and H. G. Coon, unpublished observations). It seems that they retain the necessary machinery, but for some reason they do not accumulate the polysaccharides so that they become phenotypically chondrocytes. I don't know which situation that is, but it seems to be replicability at the level of the genome.

Weiss: I am slightly concerned that no clear distinction is being made between the properties of the system we are studying and the more or less accidental signals or criteria which permit us to make a certain distinction. The pigment cell is constitutionally a pigment cell, no matter whether it is actually black or has remained uncoloured because of the lack of some terminal condition necessary for manifest production of black melanin. The pigment is not what makes the melanocyte distinct from other cells; it just makes it distinguishable. These experiments on pigment cells are as old as Doljanski's work 37 years ago; simply allowing them to proliferate will prevent them from using their protein-producing machinery for the production of melanin, by switching it instead into the competitive course of turning out another pigment cell. One finds the same thing in other cell forms where proliferation continues into adult life; thyroid cells, for instance, can use their protein either to make another thyroid cell or to produce thyroxin, but they cannot do both at the same time. There is thus a competitive situation which depends upon the environmental conditions. In short, we must not confine the term differentiation to only those characters which strike our eye or are discernible by our instruments of limited resolving power.

Paul: One restriction in Professor Abercrombie's definition of differentiation is that it is an irreversible inheritable condition of somatic cells. He mentioned a few exceptions. The exceptions are, in fact, many. In plants these are well recognized; there are also the phenomena analogous to differentiation in protozoa which are quite reversible—consider, for example, the life-cycle of *Plasmodium*.