TRANSFORMATION OF CELLS AND VIRUSES

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RECENT papers by Rhoades[1] and by Sonneborn[2], the first describing a new case of genic induction of a transmissible cytoplasmic difference (the earliest example of which is due to Imai[3]), and the second concerning a novel system of relations between the nucleus and cytoplasmic substances in heredity, are of considerable potential significance for the study of differentiation and growth, both normal and abnormal. Nowhere is this greater than in the field of cancer, where the prospect they afford of a clearer understanding of cytoplasmic inheritance must elucidate some at least of the fundamental problems involved. What these problems are, may briefly be considered.

Genetic Relationship of Normal Cells and Cancer Cells

In the entire biology of cellular variation, the transformation of normal somatic cells to cancer cells may properly be regarded as a special case, although not necessarily a unique one[4]. The alteration involves some loss of differentiation, and a nearly concomitant gain in rate of growth, the extent of which is a characteristic and permanent property of individual tumours. In ordinary conditions the change is not reversible, so that the malignant variant continues to grow, whether in the original host or on grafting to new hosts, indefinitely, without restriction, and hence with every sign of marked competitive advantage as compared with the normal form. The new cell type can be evoked at will by the use of a wide variety of physical and chemical agents (notably by X- and ultra-violet radiation, by radium and other radio-active elements, and by the carcinogenic hydrocarbons and other compounds both related and unrelated), and we now possess a considerable knowledge of their possible modes of action, of the changes they effect in the cell economy, and of the ways in which the metabolic properties of the malignant cells, in certain cases, may differ from those of their normal precursors.

Much less is known of the precise genetic relationship between the two forms. While many observers have been impressed, justifiably, by evidence which suggested the modification or loss of growth-regulating genes as a primary factor[5], these impressions are such as it is impossible to prove by ordinary genetical methods, that is, in the absence of sexual reproduction as a test. As Haldane expressed the position[6], "cancer cells do not reproduce sexually, and it is only by sexual reproduction that the geneticist can distinguish nuclear changes from plasmatic changes or virus infections".

Short of any decision, much other information, albeit of a secondary or collateral kind, or incomplete, has been derived from the study of genetic constitution as determining rates of susceptibility (whether to the spontaneous development of specific types of cancer, or to the action of carcinogenic substances), and secondly, by the production of tumours through hybridization. Thus spontaneous tumours occurring in the F_1 hybrids between *Nicotiana glauca* and *N. langsdorfii* have been ascribed to a cytoplasmic disturbance brought about by the introduction of chromosomes of *langsdorfii* into the cytoplasm of *glauca*[7]; and cross-breeding of *Mus musculus* and *M. bactrianus*, which differ widely from each other in size, fertility, and rate of growth, leads to a considerably augmented incidence of epithelial and connective tissue tumours in the first generation hybrids[8].

Although the nature of his material does not always allow the application of genetical methods, the student of cancer is nevertheless dependent on contemporary genetics to assist him in deciding which at least are the feasible mechanisms in the origin of tumours. To the present time, questions of detail have been wholly obscure, and it has always seemed likely that their solution must ultimately depend upon advances in cytogenetics as a whole. Furthermore, certain accepted characteristics of tumour cells have appeared, until comparatively recently, inexplicable and perplexing by ordinary tenets. This specially applies to the recognized irregularity of the chromosome equipment in cancer cells: while in given tumours no nuclear abnormality may be discernible, other cases present every appearance of extreme heterogeneity. In contrast with this is the fidelity and specificity with which the structural and physiological features of individual tumours are maintained, often through hundreds of transplanted generations, and apparently indefinitely. The matter has been summarized by Mohr[9]: "This pronounced uniformity of tumour tissue as regards phenotypical characteristics is just the opposite of what we would expect from the exceedingly variable chromosome relations of the tumour cells".

Cytoplasm and Growth

Considerations of this kind have led some few workers[10] to the belief that malignancy is attributable to a cytoplasmic alteration, and Koller[11] carried out an analysis of aberrant chromosome and spindle mechanism in malignant cells in an endeavour to correlate this with the behaviour of the nucleolus, which appears to hold a key position in the interrelations of nucleus and cytoplasm[12]. These anomalies have also stimulated an interest in evidence from other fields, that the more general and fundamental activities of the cell can take place even in the absence of the chromosome apparatus (if only for a time), and are governed to some extent by elements present in the maternal cytoplasm. For example, evidence has been sought[13,14,15] whether cleavage-rate in echinoderms is a function of the cytoplasm or of the nucleus. In hybridization and other experiments (with Dendraster and Strongylocentrotus) the speed of fission, in every case, was that characteristic of the cytoplasm. Secondly, E. B. Harvey's studies[16] of the growth of enucleated egg fragments (for several echinoderm species and in the annelid Chætopterus) in parthenogenetic merogony, that is, where maternal and paternal chromatin are entirely lacking, appeared to modify, or even to minimize, the role of the chromosomes and genes in early development.

Since in nearly all species the properties of the cytoplasm are controlled by the chromosomes, and because cytoplasmic factors which can be perpetuated in the absence of the appropriate chromosomes have been recognized only in exceptional cases[17] and almost entirely in plants, the problem takes the form whether the capacity of the cytoplasm to determine growth is due to a chromosomal effect persisting after removal of the nucleus, or whether it is innate and independent.

Cytoplasmic Transmission of Breast Cancer in Mice

Cognate questions arise from the so-called 'extra-chromosomal' transmission of breast cancer in mice. Since mammary cancers were found to arise with special frequencies in certain strains, attention was directed at an early stage to the presumptive importance of genetic constitution, as a factor determining the origin of such growths. The tumour-rate in hybrid strains was studied by Lathrop and Loeb[18] as long ago as 1918, when they wrote: ". . . the fact . . . that several times (but not in all cases), in reciprocal crosses, the hybrids followed the tumour rate of the mother strain, suggests the possibility that as far as the hereditary transmission of mammary cancer in mice is concerned, the mother may be more important than the father. . . ." Much later, the role of the female sex hormone was disclosed, from records of the varying incidence of cancer in virgin, breeding and ovariectomized females, by ovarian transplantation in castrate males, and by artificial administration of œstrogens to both males and females.

Further progress followed the establishment of homozygous strains, when the importance of the maternal factor was clearly established by reciprocal crosses[19]. Bittner then discovered[20] that the cytoplasmic factor is conveyed by the mother's milk, and that when young from mothers of a high-incidence line are suckled by mothers from low-incidence lines, the frequency of breast cancer in the fostered females is very considerably reduced. It is now known that the agent is present in tumour tissue, the lactating mamma, and many of the organs of high-incidence lines, and that it retains its potency in lyophilized, desiccated or glycerolated tissue, and in Seitz filtrates. Although its exact nature is not certain, it is probably a colloid of high molecular weight[21], with properties suggesting virus activity, and may seemingly arise *de novo* apart from contact[22].

According to W. S. Murray[23], the degree of mammary cancer which appears in any generation is dependent partly upon the concentration or amount of the extra-chromosomal factor which the mother transmits, and partly upon the resistance or receptiveness of animals of various genetic constitutions to this stimulus. There still remain considerable differences of opinion regarding the relative importance to be attached to the three components (cytoplasmic, nuclear, and hormonal), whether separately or in interaction. van Gulik and Korteweg[24] apparently believe that the cytoplasmic factor becomes inactive after a number of generations when a chromosomal factor is not present at the same time. But from Bittner's most recent statement[25] hormonal stimulation, inherited susceptibility (which was transmitted by males and females of cancerous stock as a dominant), and the milk agent, are of approximately equal etiological importance in mice of known constitution under normal conditions: "that is, any one of the three factors or influences may be completely determining in its effects".

Filterable Agents of Avian Tumours

A final problem concerns the induction of malignant change in normal connective tissue cells (more strictly the free histiocytes) in birds, by means of a submicroscopic and particulate agent extractable from the cells of tumours of the avian mesenchyme, of which the virus of the Rous chicken sarcoma I is the best known example. Tumours arising after inoculation of this and similar agents are derived from the prototype cells of the recipient host. They invariably conform in the minutest detail with the growth from which the agent was obtained, and they usually continue in their turn to produce further large amounts of the specific virus. In serological experiments[26] the purified Rous agent is neutralized by the serum of rabbits immunized with normal fowl serum or with normal fowl tissues, and stronger neutralization is obtained with the sera of rabbits immunized with large quantities of the purified agent itself. Further, both anti-fowl and anti-agent sera are deprived of neutralizing activity by absorption with normal chick embryo. The Rous I agent therefore appears to contain (in addition to a specific antigen) a second antigen which is also present in normal fowl tissue—a relationship which is possibly unparalleled in the whole range of animal viruses. The discovery of the Rous agent was made more than thirty years ago, and it represents one of the key observations of cancer research: yet here again it is likely that full comprehension can only be achieved through fundamental advance in other fields, such as is promised by the newer trends referred to, and the implications of which may be examined.

Cytoplasmic Determinants and their Gene-controlled Mutation

Possibly the earliest relevant observation was made by Imai[3] when he described random and irreversible mutation of a proportion of green plastids (giving green cells) to white plastids (giving white cells) in the recessive 'variegated' homozygote of barley. These plastids showed maternal transmission, and the white plastids proved autonomous and independent of nuclear control or activity, since they did not return to the green condition even under the influence of the 'green' nucleus.

Rhoades's contribution[1] concerns the gene-controlled character *iojap* in maize. Plants homozygous for the recessive gene (*ij*) develop a chlorophyll striping or variegation, interpreted as due to induction, by the gene, of modification in the plastid. Evidence is given, as for Imai's case, that the modification is irreversible, that the variant plastid possesses genetic continuity, and that this is thereafter independent of nuclear control: the mutant plastid continues to give rise to mutant plastids, in cells of whatever nuclear constitution (*ij ij*, *Ij ij*, *Ij Ij*). These relations are suggestive—and Rhoades clearly recognizes their bearing—of a mechanism whereby the expression of growth, the rate of growth, and the closely associated property of degree of differentiation, might be governed by a system of independent entities in the cytoplasm.

A more complex arrangement is revealed in Sonneborn's study of the heritable characters 'killer' and 'sensitive' in diverse races of *Paramecium aurelia*. Fluid in which the killer race has lived, kills individuals of the sensitive races, and when pure races of the two types were crossed, the two exconjugants of each pair were found to produce phenotypically different clones. It was then demonstrated that the F_1 killer clones derive their cytoplasm from the killer parent, and that the F_1 sensitive clones are those with cytoplasm from the sensitive parent. By means of technically favourable material, the phenomenon was shown to be not cytoplasmic inheritance simply, but the continued production of a cytoplasmic sub-

stance under the influence of the single gene *K*. *Addition of the cytoplasmic determinant to an organism, lacking the character dependent on it, but containing the required gene, results in the continued production of the cytoplasmic substance, in the development of the character determined by the combined presence of gene and cytoplasmic substance, and in the hereditary maintenance of the character in successive generations.*

The potential significance of these relations, both for the cytoplasmic transmission of mammary cancer and the propagation by virus of the Rous sarcoma, is sufficiently striking. They also exemplify the characteristics of cytoplasmic inheritance described by Darlington[27]: ". . . not only co-adaptation of the types of nuclear gene and plasmagene but also some degree of genotypic control in regard to the conditions of reproduction and equilibrium of the plasmagene".

Nature of the Cytoplasmic Entities: Plastogenes, Plasmagenes and Viruses

Apart from the visible plastids responsible for cytoplasmic inheritance in plants, the nature of the cytoplasmic entities remains a matter of conjecture. It is therefore reasonable to inquire what light may be thrown on cytoplasmic determiners by recent investigations of the morphological and chemical structure of protoplasm, and especially of the submicroscopic particles (microsomes) of Claude[28]. These range in size from 0·06 to 0·2 μ, and allowing for certain quantitative differences, present many similarities to the mitochondria, and appear to serve as centres for enzyme localization, both, for example, being capable of oxidizing succinic acid and giving a reaction for cytochrome oxidase[29]. Chemically, the microsomes have been found to be complex structures composed of ribose nucleoproteins and phospholipids, associated in definite proportions. By differential centrifugation, Claude isolated the active fraction from chicken tumour extracts in a form resembling fractions obtained from normal chick embryo by the same method; and he further finds that an important and possibly essential constituent of the tumour-producing particles, as of the normal microsomes, may be a nucleic acid of ribose type. The size of the Rous agent has now been determined by electron microscopy (0·07–0·1 μ), as well as in the ultracentrifuge (0·07 μ)[30]. In shape the particles are short ellipsoids, and fairly homogeneous from electrophoretic behaviour.

With the suggestion of an intrinsic origin for the avian tumour viruses may be related the view that many of the plant viruses are autocatalytic proteins of ultimate host-cell origin; and both possibilities should be compared with those different but partly relevant hypotheses which envisage many viruses arising by a process of retrograde evolution, that is, by a progressive loss of enzyme systems and synthetic functions and an increasing degree of dependence upon the cellular host[31,32]. Woods and DuBuy[33] have recently brought evidence that the characteristics of plastid-controlled variegations are intermediate between those of normal plants and virus-diseased plants, and have endeavoured to connect virus proteins phylogenetically over the variegation-inducing agents (abnormal plastids) with proteins of the normal plastids. They also attempted graft transmission of plastid-controlled variegation, which would afford direct proof of the ability of abnormal plastids to infect, and invade, previously normal cells. Although these experiments were mainly negative, such graft invasion has already been established as the cause of variegation in a number of plant species, and there is little reason to doubt that variegation-inducing plastids frequently behave like viruses, just as the plant viruses have properties often shown by plasmagenes in interspecific crosses[27]. In particular, it is likely that the changes evoked by many viruses are due to their competing for substrate with physiological elements of the cell, and thus diverting the normal metabolism. From Darlington's interpretation of Sonneborn's data[41], the 'sensitive' plasmagene in Paramecium, which is determined by the action of a nuclear gene, is suppressed by the competitive reproduction of another plasmagene. So too the variegation-inducing plastids can multiply in previously normal cells, and may restrict the development of normal plastids in those cells. The manner in which the influence of plant viruses can be likened to that of agents already present had already been noted by Stanley[34].

Induction of Heritable Change in Bacteria and Viruses

Sonneborn compared the system of determination and inheritance in Paramecium with the environmental control of genetic characters in bacteria, especially with the inter-conversion of specific types of Pneumococcus. This phenomenon was first described by Griffith[35], and it depends upon the degradation of a given specific, virulent, 'smooth' type (*S*), possessing the characteristic capsule with its specific polysaccharide antigens, into a non-specific, avirulent, 'rough' variant (*R*), lacking these features but convertible into the same or another specific and differentiated type (*S*) by growth in the presence of heat-killed *S* cells of the type to which conversion is desired. The transformation was afterwards induced by means of sterile extracts of *S* cells[36], and represents one of the most striking examples of the artificial induction of heritable change. The agent required for conversion was recognized to be not the specific polysaccharide itself, but some other component of the *S*-type cell, and Avery and his co-workers[37] have now isolated from type III pneumococci a desoxyribonucleic acid fraction which is capable of transforming unencapsulated *R* variants (derived from type II pneumococci) into fully encapsulated type III cells: the inducing substance appears to be a highly polymerized form of sodium desoxyribonucleate. It is a striking fact that the substance evoking the reaction, and the type-specific capsular substance produced in response to it, are chemically distinct. Once transformation has occurred, the newly acquired characteristics are thereafter transmitted without any further addition of the transforming agent; and from the transformed cells themselves a substance of identical activity can be recovered in amounts far in excess of that originally added, or needed, to induce the change. Assuming the transforming activity to be an inherent property of the nucleic acid, its biological specificity remains to be explained on a chemical basis. Little is known of the effects which slight differences in molecular configuration may exert on the biological action of this class of compound, although the constituent units and general structure of the nucleic acid molecule have been defined: this in itself must represent an entirely new and highly promising field.

A similar principle probably obtains for certain virus transformations. Berry[38], applying the methods discovered by Griffith, succeeded in changing the virus of rabbit fibromatosis (Shope)—in which the

lesions consist of masses of spindle-shaped cells which may recall the structure of malignant connective tissue tumours—into that of infectious myxomatosis (Sanarelli), a highly contagious disease in which tumour-like formations appear in the sub-epidermal tissues, and in which the type cell is not spindle-shaped but stellate or polygonal. In this case, however, the reaction is initiated only with difficulty, and a large excess of the transforming factors is required. A serological connexion between the fibroma and myxoma agents had already been noted, and it would seem that the immunological configuration of the killed myxoma virus particle remains sufficiently intact to provide a template for the formation of active myxoma virus, in the presence of a developing fibroma lesion. This specific mutability is a property of a considerable number of strains of fibroma virus, and the capacity of various myxoma strains to serve as transforming agents also seems to be both general and stable. The factor which induces the alteration of fibroma to myxoma virus is an integral part of the so-called elementary bodies of the latter, and Berry records a number of facts suggesting that the essential substance is the myxoma virus nucleoprotein. The transformation itself has emphasized the close relationships in a single group of viruses, which has been called the fibroma-myxoma 'spectrum', and which is capable of exciting the most widely diverse pathological effects.

Reversibility of Cellular Changes

By a few workers (for example, Dobzhansky[39]), pneumococcal transformation has been interpreted on genetic lines, the inducing substance (only later recognized as probably a desoxyribonucleate) being likened to a gene, and the capsular antigen which is produced in response to it being regarded as a gene product. The subject has also been of considerable interest to those engaged in the investigation of cancer, and Murphy[40] compared it with the virus propagation of fowl tumours, and coined the term 'transmissible mutagen' to describe the Rous and similar agents. This analogy with a mutation-producing gene is, however, only valid in a general sense, and proves less accurate in points of detail. Thus most observers have been impressed not by any resemblance of $R \rightarrow S$ transformation of pneumococci and the conversion of normal cells into malignant cells, but by the affinities of the latter process with irreversible $S \rightarrow R$ changes in bacteria. Hence, if the Rous virus corresponds with a cytoplasmic determiner, the factor inducing pneumococcal transformation conforms rather with the gene, and it converts a less differentiated cell into a highly type-specific form.

Other points of contrast arise from the differing reversibility of the two changes. Although the susceptible normal cell can easily be rendered malignant on infection with the chicken tumour virus, the tumour cell then continues to breed true, and cannot be re-converted to the normal. For certain instances of bacterial variation the R and S forms are mutually convertible, as we have seen; but in the majority, the $S \rightarrow R$ change is induced with greater facility than the reverse, and in many cases the R type is highly stable, or even permanently so. Sonneborn provides some insight into such relationships from a consideration of the relative mutation-rates, killer $\rightarrow$ sensitive, and sensitive $\rightarrow$ killer, in Paramecium. Mutations from killer to sensitive are expected more frequently than in the reverse direction, since they will occur either if the cytoplasmic factor is lost, or if the gene mutates to a form that cannot control production of the cytoplasmic factor. Contrariwise, mutation from sensitive to killer, in those cases in which the sensitive gene is present, requires both mutation of the gene and *de novo* origination of the cytoplasmic factor. Therefore, mutation in the former direction involves either of two events, while mutation in the latter direction necessitates two events in a given order.

Implications for Growth and Infection

For the larger questions of genetics and heredity, Darlington[41] has shown the significance, and the stages in its discovery, of a positive influence of the cytoplasm which is based upon unattached determinants, vested in a molecular system depending for its permanence upon a chemical rather than a morphological equilibrium, and which shows a limited capacity for independence of the mechanically stable nucleus. As he makes clear, knowledge of the plastid and cytoplasmic systems was necessarily delayed, and is only now unfolding, since it could only be interpreted in terms of a prior understanding of the nuclear system. The new conceptions are equally certain to produce their impact upon almost every other department of thought in biology, not least in the special problems of the nature of viruses, and of growth and differentiation—problems which have indeed awaited just such an advance, for their proper development.

So far as infection is concerned, it may be recalled that the progress of bacteriology itself involved a not inconsiderable readjustment of ideas. But its spectacular rise as an applied science induced in turn a prevalent unwillingness to regard any agents with biological activity of the nature of infection (and particularly the filterable viruses) as other than entirely specific and independent living organisms. In course of time there gradually accumulated a body of facts, concerned, it is true, with only a few classes of these filterable entities, such as the plant viruses and the avian tumour agents, which nevertheless appeared inconsistent with this orthodox view. Little difference of opinion has ever centred on the validity of the facts themselves: the antithesis is one of theory, and not of observation. The comprehension of anomalous cases was therefore hindered by a too limited interpretation. It is in this sense that the newer development has significance, in facilitating understanding, and on a basis sufficiently wide to include data hitherto appearing incomprehensible, or even irreconcilable. Apart from any question of identity of nature, parallels had already been drawn between the *kinetics* of gene action and virus production[42], and between the X-ray or ultra-violet inactivation curves of both viruses and genes[43]. But in certain cases similarity of behaviour becomes identity, and, for the Rous agent at least, no real distinction can be drawn between its typical activity and that of a mutant plastogene. Especially, this suggestion would account for the strict cytotropic specificity of fowl sarcoma agents, by which each transmits to the new host the characters of that particular tumour alone from which it was obtained.

Virus Etiology of Cancer in General

This broadened interpretation, valuable as it must prove to be, still affords no rationale of the curious distribution of non-cellular agents in the induction

and transmission of cancer. An agent of the Rous type may not be detected invariably, even in the Rous I tumour, its presence and absence being to some extent correlated with more rapid and less rapid growth of the cells, respectively. Other spontaneous connective tissue tumours of the fowl may completely fail to exhibit such an agent. Again, a comparable agent has not been found in any epithelial tumour, but only in those from avian mesoblast and the type cells of chicken leukæmia[44]. Finally, no such agent is present in malignant mammalian tumours, with the possible exception of leukæmia in mice. Attempts have been made to trace the source of this contrast between avian and mammalian tumours to some inherent cellular difference in the two classes. So far, the only distinction observed is a marked size-variation in avian chromosomes, the smallest particles in the metaphase plates being on the limit of resolution: this range was regarded by White[45] as characteristic of birds in general and quite unparalleled elsewhere.

Indications of infection in the natural history of cancer have rightly attracted considerable notice; but the great mass of fact shows them to be exceptional, and gives little hint of any such process as an indispensable feature of the induction of tumours. In particular, the zoological distribution of the disease—and the occurrence of somewhat analogous tumours in plants—is so wide as to lead us to suspect that the neoplastic change, by which the somatic cell, as it were, re-asserts its individuality, has in its nature something fundamental in biology, and is one to which almost every cell is liable in appropriate circumstances, quite apart from any process of infection in the bacteriological meaning, by independent and unrelated parasitic organisms. The entire evidence which might show that the avian tumour agents are independent parasites relies mainly on their unlimited capacity for multiplication in the presence of susceptible living cells either *in vivo* or *in vitro*, on the fact that cell-free filtrates of certain of the fowl tumours have been found to induce similar tumours in other avian species[46], and on analogy with proliferative although non-malignant lesions, in man and animals, caused by acknowledged viruses in the older sense[47] (for example, the pox diseases epithelioma contagiosum and molluscum contagiosum, the filterable warts of man, dogs and cattle, and infectious papillomatosis of rabbits). The last comparisons are admittedly compelling, but the other criteria have proved less easy to maintain. The first (capacity for multiplication) can clearly no longer be accepted as necessarily attesting the living or extrinsic nature of the material undergoing increase.

Significance has also been attached to the transmission of the Fujinami fowl myxosarcoma to ducks and of the Rous I sarcoma and a fowl endothelioma by filtrate to pheasants, but it is pertinent[48] that transmission in both of these cases is still within the limits of blood relationship as judged by the precipitin reaction: moreover, propagation to the pheasant is within the limits not only of blood relationship but also of bastardization. (Apart from modern examples, it is of interest that Darwin made reference to *phasianus* × *gallus* hybrids in Chapter 9 of the "Origin of Species".) The successful transmission of a fowl tumour to pheasant or duck does not therefore make it less likely that the filterable agent is a cell-derivative on one hand, or more likely that it is an independent parasitic virus on the other.

The discovery of the plasmagene would have held a special interest for Boycott, who always found it difficult to escape the conclusion that the Rous agent arises intrinsically and *de novo*, and who said on a memorable occasion, "if one postulates a normal virus occurring in normal cells, one had better call it something other than a virus"[49].

Cytoplasm and Differentiation

Both Rhoades and Sonneborn are aware of the implication of their findings for differentiation, in explanation of the fact that while all the cells of an organism presumably have the same genetic constitution in the nucleus, they nevertheless exhibit wide morphological and physiological differences, which are not entirely due to differences in tissue environment: and the view is put forward[1] that cellular differentiation is determined by hypothetical particles in the cytoplasm. The production of different characters in cells with the same nuclear genes would thus be brought about by differential segregation of these cytoplasmic determiners at cell division[2] in a manner similar to that which governs the segregation of plastids. As has been emphasized, the question of differentiation is paramount in the study of cancer. The cells of a given tumour usually show some degree of structural and functional affinity to their normal parent cells: so a cancer of the breast may possess a glandular structure obviously related to the architecture of the normal organ, the cells of a cancer of the liver may retain a considerable degree of resemblance to normal liver cells, and tumours of secretory organs may continue to elaborate the characteristic product, whether hormone or enzyme, of their normal prototype. In general, however, the change from a normal to a malignant cell connotes some loss of special functions, and the tumours of a given lineage can be placed in a continuous series ranging from a near-perfect reproduction of the histology of the parent tissue to a condition in which no specific differentiation can be recognized whatever[50]. The extent of such departure is relatively stable for any given tumour, and the greater it is, the nearer (in many cases but by no means invariably) does the new cell tend to approach an embryonic type and the greater is its rate of growth.

These old and new facts, taken together, suggest a means whereby light may be shed on the central problem of the mode of action of carcinogenic agents other than virus-like influences; that is, the chemical carcinogens obtained by synthesis or from sources outside the body. In our approach to this problem one salient fact overshadows all others: that the growth of the tumour is not in any sense dependent upon the continued presence of the agent which provoked it. In other words, the chemical carcinogen produces a change in the habit of growth of the cell, but as the change is quite permanent, it persists indefinitely after the initial cause has disappeared or has been removed. It is patent that the carcinogen does not provide the real stimulus to growth, since growth proceeds without it. Hence the mechanism which permits unlimited growth must clearly reside in the cell itself. That it may reside partly at least in the cytoplasm, and conceivably in relation to the considerable quantities of ribose nucleic acids which the studies of Brachet, Caspersson and others have shown to be present there when rapid synthesis is taking place, is obviously a possibility for future investigation, both by cytological methods and by experiment.

The new discoveries have, therefore, the widest implications, and for specialized matters no less than for those of a more general nature. In many cases the relationships seem more than mere analogies, and strongly suggest an underlying unity of principle in the growth and differentiation of organisms of the most highly diverse kinds. They also testify to the particular value, notwithstanding its recognized limitations, of the study of variation in unicellular organisms, and sustain the belief, long held by Dobell and others, and now more widely shared, that more than one of the current conceptions in biology must undergo profound modification as a result.

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RECENT DEVELOPMENTS IN POLAROGRAPHIC ANALYSIS

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ALTHOUGH almost twenty years have passed since the polarograph was devised by Prof. J. Heyrovský and his colleagues at the Charles University in Prague, it has only been during the last two years that use of the instrument has been widely accepted by industrial laboratories in Great Britain. Nevertheless, it is now generally agreed that the instrument is of unquestionable value, and the polarograph is taking its place in the equipment of the modern laboratory along with the spectrometer and photo-electric absorptiometer.

The fundamental principles of polarographic analysis were worked out in Prof. Heyrovský's laboratory and have since been confirmed in the United States. These principles are fully described in an excellent monograph by Kolthoff and Lingane[1] which surveys the literature of polarography up to the end of 1940. Since that date, many new applications have been developed, especially in the biological and organic fields, and it is these that I propose to survey.

Polarographic analysis depends essentially on the fact that when a gradually increasing potential is applied to an electrolyte solution in a special cell consisting of a dropping mercury electrode and a second non-polarizable electrode, it is possible to determine from the resulting current-voltage curve both the nature and the concentration of the reducible or oxidizable substance or substances present. It is these current-voltage curves that are recorded by the polarograph.

A typical polarogram obtained with an air-free solution of 0·001 *M* cadmium chloride in 0·1 *N* potassium chloride is shown in Fig. 1. Under standard conditions, the limiting or diffusion current (that is, the height of the step) is proportional to the concentration of the electroreducible substance. This serves as the basis of quantitative polarography. The half-wave potential, which, as its name implies, is the value of the potential of the dropping mercury electrode, standardized against an external reference electrode (usually the saturated calomel electrode), at that point on the current-voltage curve when the current is one-half its limiting value, is a special property of the particular electroreducible substance present and is independent of the concentration of

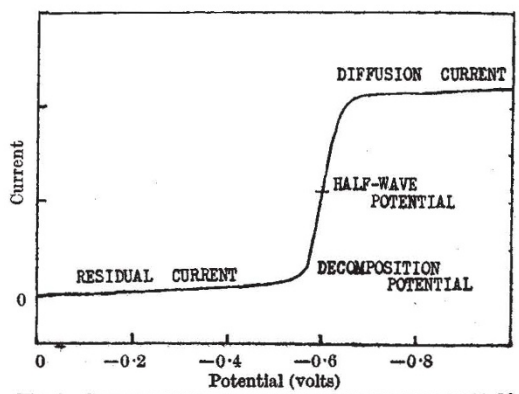

Fig. 1. CURRENT-VOLTAGE CURVE FOR SOLUTION OF 0·001 *M* CADMIUM CHLORIDE IN 0·1 *N* POTASSIUM CHLORIDE.