

## Cell Genetics and Hereditary Symbiosis

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RECENT SYMPOSIA HAVE been attended by controversies over the interpretation and scope of cytoplasmic inheritance, often centering on the diagnosis of virus *vs.* plasmagene (5, 42, 43, 45, 48, 55, 197). This review is an attempt to reconcile these views and, where possible, to look for further implications of the synthesis. This account is (quite properly) limited by space allowances. Therefore, objective reviews which already survey the experiments will be cited, when available, in lieu of original references. The treatment of material which has not been condensed previously is therefore exaggerated. An eclectic construction from the freely borrowed views of many authors has been attempted, and few of the interpretations are original.

The problems of cytoplasmic heredity have often merged with those of embryonic development and somatic differentiation (56, 115, 125, 182, 183, 202, 203, 212, 220). The genetics of somatic cells has, however, been poorly studied. Their constitution has been deduced from that of the zygote or the whole organism, except for a few, but important, studies on somatic mutation (66, 119, 123) and somatic segregation (83, 88, 189). Cytological studies have taken some exceptions to the genetic uniformity of somatic cells of the differentiated organism, but have not thereby contributed affirmatively to a comprehensive theory of development (83, 121, 220). Although the pertinence of extranuclear heredity for ontogeny has been generally accepted, in the absence of extensive evidence for it, plausible theories of development have been constructed which rely on the ultimate primacy of the nucleus (73, 131, 193, 202, 220). For technical reasons, cell genetics is best studied in organisms whose germ and soma are not irreversibly differentiated, especially in the microorganisms where vegetative proliferation is preeminent. Whether methodological obstacles with extranuclear heredity factors exaggerate the seeming dominant role of the nucleus in the genetics of higher animals and plants has been debated elsewhere (37, 55, 90, 131, 181, 220).

These discussions have left a plethora of terms adrift: pangenes, bioblasts, plasmagenes, plastogenes, chondriogenes, cytogenes and proviruses, which have lost their original utility owing to the accretion of vague or contradictory connotations. At the risk of adding to this list, I propose *plasmid* as a generic term for any extra-chromosomal hereditary determinant. The plasmid itself may be genetically simple or complex. On occasion, the nuclear reference of the general term *gene* will be emphasized as *chromogene* (219).

This review is dedicated to the reconciliation of the attitudes that plasmids are symbiotic organisms, and that they comprise part of the genetic determination of the organic whole. The conflict may arise in part from fixed conceptions of the scope of the organism. Heuristically, the taxonomic classification of plasmids as viruses, symbionts, or plasmagenes should not obscure careful descriptions of their function, hereditary or pathological, or both. This viewpoint need not prejudice

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the evolutionary origin of different plasmids, although it may suggest possibilities of wider connections.

#### GENETIC CONTINUITY OF CYTOPLASMIC ORGANELLES

Various structures that are characteristic of almost all cells have been suspected of hereditary functions from morphological evidence of their genetic continuity. The history of the nucleus in gametogenesis and fertilization, and the constant number and individuality of the chromosomes convinced many cytologists of their role in heredity well before the rediscovery of Mendel's work (55, 211, 212). However, it was not until the parallelism in the distribution of the chromosomes and the chromogenes was affirmed even for exceptional deviations (129) that their identification convinced most biologists. No extra-chromosomal organelle has met this criterion except in the most rudimentary way. Within the nucleus, the nucleolus has been thought to be continuous but is now regarded as a metabolic product concentrated at a special chromosome region (38, 122). The nuclear membrane is continuous throughout mitosis in many protista, but has not exhibited any of the differentiation needed for genetic analysis.

The mitochondria have stimulated the greatest interest, but little has been added to our conception of their genetic functions in animal cells since Wilson's discussions over twenty-five years ago (135, 213, 214). Their development during spermatogenesis in scorpions has furnished compelling suggestions but no proof of their role in heredity. In *Centrurus* the mitochondria aggregate in the spermatocyte to form a single large ring which is accurately partitioned at meiosis. In *Opisthacanthus*, twenty-four spheroids are formed which are equally distributed, six to a spermatid. Despite his contrary conclusion, Wilson's statistics show that the distribution is more precise than random. The small size and great number of the mitochondria have made it very difficult to study their morphogenesis in somatic cells. The genetic relationship of mitochondria to the submicroscopic microsomes is still controversial; part of the life history of a mitochondrion may range beyond microscopic visibility to be confused with *de novo* origin. Our appreciation of the chemical constitution and enzymatic activity of cellular particulates as a whole (40, 75, 135, 168, 213) is more advanced than their taxonomy. The distribution of properties among the particles collectively labelled mitochondria is an urgent question for the geneticist.

Many workers early described the origination of mitochondria from nuclear fragments—the chromidial theory now largely discredited (135). Current opinion emphasizes the continuous functioning of the energetic nucleus (38, 73, 143, 190) rather than an intermittent outpouring at karyokinesis or karyolysis. The chromidia have a counterpart in gene-initiated but autonomous plasmagenes, postulated as one mechanism of nuclear control (45, 219, 220), but this theory has not been substantiated to the exclusion of nuclear regulation (rather than origination) of pre-existing plasmids.

The genetic continuity of other cytoplasmic inclusions is even less well verified than that of the mitochondria. The chloroplast, often regarded as a mitochondrial derivative, will be reviewed in detail below.

Special attention has lately been given to the fiber-organizing granules which function as centrioles, blepharoplasts, parabasal bodies, basal granules etc. (115), for which the general name *kinetosome* has been suggested. Although different kinetosomes may serve specialized functions in the same cell, several substitutions of function have been described. For example, the kinetochores of degenerating chromosomes

of apyrene spermatids in viviparid snails become detached to function as accessory blepharoplasts in the development of multiflagellated spermatozoa (145). However, the spermatogenous mitoses of mosses and ferns, where centrioles and blepharoplasts appear to arise *de novo* (212), raise the question of the strict genetic continuity, or at least the individuality of the kinetosomes generally. Although the organized kinetosome system of a ciliate may function in morphogenesis as a community of mutually regulatory, but individually autonomous parts (80, 115, 202, 203), their ultimate potential origin from a common indifferent reservoir is not excluded. The best evidence of the individual autonomy of a kinetosome is the formation of apara-basal trypanosomes when its division is impaired by trypaflavine or colchicine (144). More work needs to be done on the details of this impairment, but the loss appears to be strictly irreversible. A possible direction for more detailed consideration of the kinetosome (which is frequently reported to be Feulgen positive) as genetic units is suggested by a *Drosophila* mutant in which kinetochore action is impaired, though not abolished (9).

#### CYTOPLASMIC INHERITANCE

The profusion of terms nearly equivalent to plasmid already points to the divergence of the morphological and genetic exploration of the extra-nuclear territory. The literature on cytoplasmic inheritance has been recently and critically reviewed elsewhere (13, 37, 42, 43, 45, 55, 56, 115, 125, 181, 182, 219, 220) so that only a few of the most illustrative studies need to be restated here. Cytoplasmic and extranuclear are misleading locatives. A resident of the nuclear sap (e.g. 199) would often be genetically transmitted no differently from a cytoplasmic factor. In fact, supernumerary chromosomes whose segregation is irregular, and may differ in oö- and spermatogenesis, might give genetic results readily mistaken for cytoplasmic transmission. The concept of the plasmid is meaningful only in terms of regular, well-understood nuclear behavior, and various claims of nonmendelian inheritance must be re-evaluated on this criterion (106, 107, 162, 216).

#### CHLOROPLASTS: PLASTOGENES AND VIRUSES

The most extensive literature on extranuclear inheritance concerns the chloroplasts of seed plants. Many mutants affect the chloroplasts and the synthesis of chlorophyll, but most are recessive lethal or semi-lethal mendelian factors (76, 159, 172, 200). We shall deal here with variants that show maternal or matroclinous inheritance, so that plasmids are implicated (157, 159).

Morphological evidence of chloroplast continuity is inconclusive (135). Mature plastids may divide as such, or may develop from mitochondrial primordia in meristematic cells. The genetic duality of the mitochondria and pro-plastids is controversial and not yet supported by morphological discrimination. Plastid genetics has mainly dealt with plastid variegations, some of which resemble virus diseases (218). Affected plants show a regular or irregular mosaic of normal and chlorotic tissue. Seed from the white shoots will usually give moribund white seedlings, whereas the pollen may function normally to give standard green progeny when crossed to normal green. The demonstration of virus infection by mechanical or graft transmission to healthy plants has disqualified a few cases originally thought to belong in this group (19). The plastid variegations have been interpreted by many workers as indicating a segregation of mutant white plastids, which arise from a highly unstable green plastid condition. As a rule, however, the green and white plastids are not well de-

lineated in single cells, and there may even be a transition zone several cells deep at the margin of white patches in which the plastid condition is intermediate. Other workers have therefore concluded that the variegations are not inherent in the plastids but in another element of the cytoplasm. The term *plastogene* was introduced to describe the potentially mutable genetic unit of the plastid according to the first hypothesis, but will be useful for present discussion to denote the determinant of plastid behavior, whatever its form or location in the cytoplasm (84, 159, 200, 218).

To restate the problem, are the plastogenes confined to the plastids (hypothesis of genetic autonomy) or distributed independently in the cytoplasm (a generalization of the second hypothesis)? Are the plastogenes elementary units or would it be profitable to investigate their genetic complexity by combination experiments? If the plastogene is located on the plastid itself, is it also autonomous in function, or is the phenotype of all the plastids in one cell regulated as a whole by the plastogene aggregate? The last consideration weakens the force of the blending of plastid phenotypes within single cells where this occurs (but see 218).

The stability as well as the function of the plastid system is under chromogenic control as shown by recessive genes which initiate plastid variegation (84, 159, 218). In maize, for example, homozygous *iojap* plants, obtained by selfing normal green heterozygotes, show a marked plastid instability that is reflected in a green and white mosaic. By further crossings, the *iojap* can be replaced by wild type genes, but the modified plastid character persists in maternal inheritance. Catnip (*Nepeta*) may be more favorable material for similar studies, because it can be kept indefinitely by vegetative propagation, and self-inviabile types can be grafted to green stocks. Woods and duBuy have described a wide range of qualitatively distinguishable plastid types which develop under the influence of a recessive chromogene analogous to *iojap* (218). These authors have postulated hereditary modification of the plastid primordia (chondriogenes) for the origin of plastid variegations and transmissible viruses. However, the effective plastogenes have not been conclusively located in the affected plastids. In other terms, we might say that a previously latent virus (with the remarkable property of being cell-bound but seed-transmitted) had been activated by the *iojap* genotype so as to modify the character of the plastids. It is feasible to take advantage of the occasional transmission of plastid primordia through the pollen to show that green plastids will recur in seedlings from *white* x *green* purified and maintained by grafting on *green*. The *white* x *white* cross would serve as the necessary control. The technical features of this material also permit the combination of different plasmid genotypes.

Chloroplast mutants have also been described in irradiated material, of which fern prothallia have been most studied (120). Unfortunately, a genetic analysis has not been reported, so that the site of the mutation is not known.

Experiments with the chloroplasts or chromatophores of certain algae support their genetic autonomy. In *Euglena* species, certain conditions of cultivation in the dark on rich media led to a diminution in the chromatophores, occasionally followed by their irreversible loss (114). However, the affected clones may have been intrinsically unstable, and the conditions of culture merely permitted the survival of the apochromatophoric clones (152).

Chemotherapy is a promising new tool of plastid and plasmid research. Attempts to remove bacterial contaminants from cultures of *Euglena gracilis* with streptomycin resulted instead in curing the flagellate of its chloroplasts (154). In some strains the disappearance of the chromatophores was accompanied by that of the carotenoid

eyespot or stigma; in others, the stigma persisted, but only so long as the culture was illuminated. Once lost, neither chromatophores nor stigmata were restored, even upon prolonged cultivation in the light. The success of this experiment depends, of course, on the heterotrophic potency of the *Euglena*. Other algae are bleached, but cannot survive without photosynthesis. In still further species, other functions are more sensitive to streptomycin, and their inactivation precedes apochlorosis. The fate of the pyrenoid and its relationship to the mitochondrial system of the bleached cells are controverted (116, 152).

Unfortunately, sexual reproduction has not been described in *Euglena*, precluding detailed genetic investigation, but other algae are under study. The similarity between the artificial apochlorotic variants and naturally occurring *Astasia* has given point to hypotheses proposed for the origin of various colorless flagellates (114, 152). Apochlorosis has also been achieved in some *Euglena* strains by temperature treatments (153). The conditions suggest that the chromatophores are lost by not keeping pace with cell division, rather than by their active destruction, as with streptomycin.

Some seed plants are also susceptible to streptomycin. As albino shoots can be maintained in mosaics with or by grafts on green tissues, there are extensive possibilities for genetic study with such material. Together with the plastid variegations, these studies have already provided considerable evidence for the genetic autonomy of the chloroplast. Such evidence is, however, by no means conclusive, and other plasmids, not yet characterized, may be immediately responsible for the observed effects.

Male sterility is maternally inherited in many plants. The pathology of pollen abortion hints at a virus which might be transmissible, but despite its economic value (89, 158), this has not been achieved. Unlike the plastid variegations, the male sterility factor of maize does not sort out in somatic tissue, as shown by the absence of chimaeras on the ears, and by a biometric study of the pollen of partly affected plants (67, 158). Segregation does occur during or soon before microsporogenesis (cf. the aggregation of scorpion chondriospheres, 214) so that some microspores escape the plasmid and do not abort. The expression of male sterility and the maintenance of the plasmid are under rather complex chromogenic control (173, 89). Two recurrences of the male-sterility plasmid have been reported in genetically controlled maize cultures, one of them in an *iojap* genotype (160, 173). Its origin by mutation of normal plasmids (mitochondria) has therefore been proposed, quite plausibly. However, to the reviewer's knowledge, an infectious origin under field conditions has not been explicitly disqualified.

#### CYTOPLASMIC MUTATION IN YEAST

Dwarf colony types had been sporadically observed in yeast cultures for many years, but until Ephrussi's studies (57) little attention was paid to them. In the course of experiments with chemical mutagens, it was noted that yeast suspensions exposed to euflavine (2,8-diamino-10-methylacridine) produced large numbers of 'petites colonies' (PC). Despite the occasional spontaneous occurrence of the PC, a careful population analysis, and later the direct examination of daughter buds from treated cells showed unequivocally that the PC 'mutation' was specifically, directly, and prolifically induced by the acriflavine. The suspicion that the mutation recurred in a cytoplasmic rather than nuclear particle was reinforced by the equal effect of euflavine on haploid and diploid yeasts and verified by crosses of PC with wild type. Such crosses regularly restore wild type zygotes whose further progeny is also nor-

mal, implying that the PC mutation occurs when a plasmid is irreversibly lost, as accelerated by acridine dyes. This depletion is not a direct destruction of the plasmid: treated mother cells remained normal for some time after they had engendered a series of PC buds. Instead, the transmission of the plasmid is probably primarily affected, although it may subsequently be directly modified in the mother cell. Since euflavine is known to precipitate nucleic acids and agglutinate bacteria, it may aggregate the plasmids or otherwise hinder their passage to the bud. Limited attempts at artificial re-introduction of the wild type plasmids into PC cells have been unsuccessful.

The dwarf growth of the PC is a consequence of the lack of cytochrome oxidase and succinoxidase associated with mitochondria. In the wild type yeast, some of the 'mitochondria' give a positive indophenoloxidase (Nadi) reaction; in the PC they are all negative. This plasmid can therefore be plausibly considered as a genetically self-determined set of mitochondria carrying the specified enzymes but, as Ephrussi himself has cautioned, the evidence for the identification is inconclusive. The plasmid might correspond to a distinct cytoplasmic element necessary for the elaboration of the oxidases, which are localized on various mitochondria. This need not be a gratuitous multiplication of determinants: The same phenotype is controlled by a chromosome in a segregational PC mutant, but one does not identify the nuclear gene with the oxidase system. In fact, the enzymes themselves are certainly not self-perpetuating as such, for dormant plasmids remain intact in the segregational PC mutant, ready to co-act with a suitable gene substitution to redevelop the oxidase system. Thus, the copulation of a segregational PC with a vegetative PC mutant results in a wild type zygote.

An attractive theory of chemical carcinogenesis has been constructed on a similar basis (148). The liver of normal rats contains a protein that reacts with aminoazobenzene derivatives. Feeding on these dyes depletes the dye-binding protein, together with succinoxidase activity and the microscopically visible mitochondria. The initiation of tumors is correlated with the total depletion of the reactive system. Potter has speculated that a diversion of high-energy metabolites from regulated physiological functions leads to unrestrained growth.

Mitochondria are now a favored subject of biochemical research, with accumulated evidence that biological oxidation sequences are coordinated in granules, corresponding to some or all of the mitochondria (40, 75, 135, 168). The metabolic pattern of mitochondria differs from tissue to tissue in the same organism. Whether this differentiation involves the quality of individual granules, or the distribution of diverse species is not known. The prospects for experimental study of genetic differentiations of mitochondria are limited with material whose vitality depends on the integrity of the mitochondrial system. The PC mutation would certainly be lethal in fruit fly or mouse and a similar argument might account for the paucity of other plasmid demonstrations in higher forms. If induced hepatomas are viable cells analogous to PC mutants, however, the door to such studies with animal cells is not closed, and cytochemical specialists should be encouraged to correlate their work with genetic studies.

The slow adaptation of certain yeast strains to galactose straddles the persistence of genetic variation and the transience of physiological response. The understanding of long-term adaptation has been confused by several different mechanisms with superficially similar effects. The common datum is that various yeast strains, which do not immediately ferment galactose, acquire the ability to ferment this sugar

over a period of several days. Adapted inocula, when maintained in the presence of galactose, develop into promptly fermenting cultures (184, 215). One yeast strain becomes stably adapted after variable intervals of exposure to galactose. This adaptation almost certainly consists of the selection of spontaneous mutants.

We are more concerned with other strains which respond to galactose after a constant interval, and whose adaptation disappears abruptly when the cells are exposed to glucose. The de-adaptation has been analyzed both in mass cultures and in single buds, with results that leave no doubt of the direct effect of the glucose, as opposed to natural selection (184). But, unlike the PC account, the mother cell is as likely to be de-adapted as its bud. The kinetics agree with a model in which the adapted cell carried about 100 plasmids, distributed at random to bud and mother. A single plasmid will propagate in the presence of galactose until the normal level is attained. Without galactose, the plasmids remain dormant and are passively apportioned to successive generations, until the average number per cell is too low to assure that the next generation will be uniformly infected. As might be surmised from the kinetic differences, these plasmids are distinct from those affected in the PC mutants, and from the galactozymase complex itself, which disappears much faster than adaptability. Other evidence also makes it very unlikely that an enzyme is itself part of the mechanism of its adaptive formation (103, 128, 186).

The chief inadequacies of this theory are that it does not encompass the original mechanism of slow adaptation to galactose, and that this has not been resolved in terms of individual enzymes. In populations grown on glucose, about one cell per thousand eventually responds to galactose. Competent plasmids might arise *de novo*, i.e., from the nucleus or from other plasmids, but this *ad hoc* assumption should eventually be replaced by more precise testable specifications. The kinetic studies of de-adaptation are, however, consistent with the plasmid-depletion theory which is tenable in the absence of clearly formulated alternatives.

Further study of the biochemical competences of individual cells would be fruitful for embryological analogies, but very little is known of the physiological fluctuations in adaptability of microorganisms. The ability of *Aerobacter aerogenes* to assimilate citric acid, presumably some sort of adaptive enzyme process, is physiologically variable from cell to cell, and responsive to nonheritable inactivation by ultraviolet light (155). Similarly, the inhibition of enzymatic adaption of *Pseudomonas fluorescens* by ultraviolet light, and its restoration with visible light, might be interpreted in terms of an all-or-none response of individual cells (186). Unfortunately, few systems are technically suitable for determining the adaptive status of a single cell, although more use might well be made of microculture methods.

#### CYTOPLASMIC INHERITANCE IN PARAMECIUM

Genetic studies with the relatively large animal, *Paramecium aurelia*, are hardly at all complicated by the populational selection difficulties sometimes encountered with other material, but there is, unfortunately, a dearth of mutant chromogene markers, especially for the biochemical characters which have been so fruitful with the microthallophytes. Sonneborn and his co-workers have not permitted this difficulty to obstruct their painstaking study of the nuclear and cytoplasmic hereditary systems (178).

The most distinctive cytological feature of most ciliates, including *Paramecium*, is the dual nuclear system (179, 203). The micronucleus which alone is involved in fertilization and meiosis, but is propagated mitotically during clonal growth, has

been compared with the germ line; the macronucleus which is derived anew from the micronucleus after each fertilization, and whose fission is obscure, with the soma of metazoans. The somatic function of the macronucleus is seen by its regulation of all gene-controlled functions during clonal proliferation in amiconucleate or allo-karyotic animals. As a rule, the macronucleus is derived from the micronucleus at the last preceding reorganization, and will be genetically equivalent to it. Under certain conditions, or in the absence of a micronucleus, the macronucleus will be regenerated from fragments of the previous macronucleus, irrespective of the genotype of the current *micronucleus*. The two nuclei may also come to deviate by mutation. *Paramecium* is extraordinarily resistant to ionizing radiations, but x-rayed or nitrogen mustard-treated lines may show a high mortality at the next nuclear reorganization. Much of this delayed effect may be accounted for by lethal mutations or chromosome deletions whose phenotypic effect is masked by the macronucleus as well as by dominance in the micronucleus. Finally, differentiation within the macronucleus has been described in other ciliates as a response to organizing fields in the cytoplasm (203).

To the extent that the macronucleus may be regenerated from fragments of its predecessor it becomes genetically independent of the micronucleus, and might even be regarded as a giant plasmid. Macronuclear fragments have even been observed, on occasion, to be transmitted to conjugant partners. As a rule, the micronucleus retains its ascendance, but the persisting macronucleus is the most concrete example of the gene-initiated plasmid. The hypothetical origin of plasmids from macronuclear fragments recalls the chromidial theory but merits consideration on its own merits.

Clones of *P. aurelia* vary in the production of a poison, *paramecin*, and their sensitivity to it. Experimental determination of its genetic control is facilitated by the conjugation mechanism. The reciprocal exchange of gametes makes the two conjugants identical in their chromogenes, but each animal retains the intact cytoplasm of its progenitor. When separation of the conjugants is experimentally delayed, however, some cytoplasm is exchanged. Two genetic requirements for the killer or paramecin-producing character were discovered: 1) that the animal carry one or two doses of a gene K, i.e. that it be KK or Kk, and 2) that the K-bearing animal have received cytoplasm from a killer animal, from which a plasmid  $\kappa$  is inferred.  $\kappa$  was once thought to be very intimately related to K, i.e. that it was either initiated by K or participated with it in a bipartite unit in the macronucleus, but these hypotheses have since been abandoned as unnecessarily elaborate. The results are best summarized as dependence of the maintenance of  $\kappa$  on the chromogene K. This relationship is perhaps nutritional: The quantitative level of  $\kappa$  is proportional to the number of K genes in a given genotype, but the substitution (by autogamy) of a kk for a Kk macronucleus causes a depletion of  $\kappa$  only after a considerable interval.

Paramecin and  $\kappa$  both contain desoxyribonucleic acid as an essential component. However, KK and kk animals (sensitive for lack of  $\kappa$ ) are equally sensitive to paramecin, and no proliferation of the paramecin has been detected. The distinction of  $\kappa$  and paramecin bolsters the critique of premature identification of plasmids and their products in other systems.

Evidence of the genetic autonomy of  $\kappa$  has come from the study of mutants affecting paramecin production. In a number of such mutants, variant  $\kappa$  was found, affecting both the quality and amount of paramecin. Additional alleles of the chromogene K have not, however, been described (50). Following upon the morphological



demonstration of  $\kappa$ , its further study has followed a new course. It will therefore be resumed under endosymbioses.

A second system of cytoplasmic determination in *Paramecium* affects the ciliary antigens (180-182). When paramecia are injected into rabbits, antibodies are produced which paralyze the cilia and kill the paramecia if the treatment is too intense or prolonged. The different antisera constitute a series of reagents *A, B, C* . . . which react strongly with some animals, and not others, thus permitting a serotypic classification.

The first evidence of extranuclear determination is the inter-transformability of serotypes, which is especially dramatic in homozygous animals exposed to specific reagents. For example, stock 51, *variety A*, shows three frequent serotypes, *A, B*, and *D*, each of which is clonally stable. However, an animal exposed to liminal concentrations of its antiserum becomes converted to one of the other types, establishing a clone of equally stable serotype. The serotypic transformation is reversible so that all types are mutually inter-transformable. Under 'standard' conditions, the existing type is perpetuated, unless disturbed with antiserum, and each type has equal stability. Under other conditions of temperature and nutrition, however, one type is preferred, and all others spontaneously transform to it. Even the antiserum effect may be indirect, and result in part from the temporary alteration of growth habit induced by the antiserum (175). The cytoplasmic basis of the serotype is verified by mating experiments: each exconjugant breeds true to its original serotype under standard conditions.

The interplay of chromogenes with cytoplasm is revealed by comparisons of stocks which differ principally in the range of serotypes detected in transformation experiments. Thus stock 29 produced a serotype *F* not characteristic of stock 51. Crossing experiments showed that the potentiality (or range of conditions) of transformation to *F* was controlled by a dominant chromogene. Minor modifications of the *A* antigen are also controlled by chromogenes. The genes thus discovered have been termed 'specificity genes' controlling the different antigens, but it may be more circumspect to picture them operationally: the chromogenes control the conditions under which the cell may produce a particular range of antigens.

In *variety I*, genetically homologous antigens, i.e. antigens controlled by allelic genes, may be serologically unrelated, and both alternatives displayed in the same heterozygous animal whereas nonhomologous serotypes are mutually exclusive. Thus, "only genes at one of the three loci are normally expressed at a given time . . . depending on the state of the cytoplasm." (15). This does not mean that the other genes are inert (a naive fallacy still voiced too frequently) but that the different alleles do not alter the serotype of the preferred cytoplasmic state. The results with *variety I* show that it is the cytoplasmic states that are mutually exclusive, and that the antigens are a product of the co-action of the chromogenes and the cytoplasmic state.

The persistence of the existing cytoplasmic state may be interpreted in seemingly different ways. Each state might correspond to the predominance of particular plasmids, the populations of which compete for nutrients. In order to account for the reversibility of serotypic transformations, however, some means of replenishment of different plasmid types would have to be postulated. Suggestions include re-initiation from the macronucleus, the persistence of specific plasmids at low concentrations, or a *de novo* origin by mutation of other plasmids. An alternative hypothesis dispenses with particulate plasmids, but refers to anabolic steady states. Alternative reaction

sequences from different genes to final antigens are supposed to compete for metabolites. The stability of the existing state is explained by regenerative feedback whereby a given product facilitates its own synthesis, or inhibits alternatives.

These hypotheses are less distinctive than may appear at first sight. Any plasmid behaves in much the same way. For example,  $\kappa$  can be regarded as a gene product, in view of its dependence on the K chromogene.  $\kappa$  differs from the serotypic states insofar as it can be irreversibly suppressed, whereas the serotypic states remain mutually transformable. However, this may merely reflect the greater complexity, and concomitant autonomy, of  $\kappa$  which make it unlikely that it can be readily revolved from non- $\kappa$  cell constituents. The elementary autocatalytic reactions of growth that underly the reproduction of any organism are subsumed under the steady-state hypothesis. Further investigation may furnish more details of the cytoplasmic states, but are unlikely to decide between tautological hypotheses. Because of its simplicity, the antigen system may perhaps furnish information on genetic conservation and growth that is less readily obtained from more complex experimental materials. Cytoplasmic states clearly parallel the stable states of developmental modulations and differentiations. The mechanisms that may be involved in embryology or neoplasia are best explored, at first, in this type of model. The actual processes can only be told by looking at the embryo or tumor itself.

A convergence of this model with original is furnished by an account of pattern differentiation in spotted mammalian skin (17). Pigmented areas, at first sharply delineated, tend to spread as the spotted guinea-pig or bovine matures. The spreading is exaggerated when pigmented skin is autografted to white. It might be explained by the migration of pigmented dendritic cells (melanophores), and their replacement of unpigmented cells. Alternatively, pigmented cells are thought to inoculate a melanogenic plasmid into unpigmented dendritic cells with whose terminal processes they fuse. Homograft pigmentation also succeeds but only when the donor seeds are so small as to avert iso-immune reactions (176). The experiments still do not entirely disqualify the cell migration hypothesis, if we impute to the melanophores the same low immunogenicity but high sensitivity to antibodies as claimed for the postulated plasmids. Either melanophores or plasmids must differ from other tissue elements which quickly provoke the histo-incompatibility reaction in homografts. Even on the plasmid or cytochrine hypothesis, the identification of the plasmid with the visible melanin granules (presumably mitochondrial derivatives) is tempting but so far unsupported. If verified, the plasmid interpretation would be the first concrete justification of the general theories of somatic differentiation based on the assortment of plasmagenes.

The genetic basis of the hereditary, irreversible differentiations of somatic cells is still obscure and experimentally difficult (125, 202). With mammalian tissues, there are few cellular characters that can be related to genotypic alterations observable in crosses of intact animals. However, promising material is available in the histo-compatibility patterns of transplantable tumors. A number of histo-compatibility variations have been described in clones of transplanted tumors, and it should be possible to relate them to mutations of specific loci by immunological tests (108, 119, 176, 192). Although their origination as spontaneous mutations is most plausible, there are some obscurities which require further study to ensure that the histo-compatibility (or iso-antigenic) factors are directly involved, especially as virus contaminations may influence the growth of a transplant (194). The possibility of detecting somatic segregation in tumor cells originating from heterozygous animals should not

be overlooked. Although a microbiological approach to the genetics of tissue cells is still technically difficult, demonstrations such as the spontaneous origin of mutations for resistance to anti-leukemic chemicals (98) afford high promise. These mutations are not developmental differentiations, but their study may be expected to lead to the tools needed for the genetic study of development per se.

#### GENETIC TRANSDUCTIONS (TRANSFORMATIONS) IN BACTERIA

Genetic transfers in bacteria have been apprehended to infective heredity by many authors including the reviewer (10, 45, 102, 177). The first well-authenticated transduction was the type transformation of the pneumococcus. Purified extracts of capsulated cultures induce stable homologous capsulated strains from uncapsulated variants. Analogies were soon suggested to the transmission of latent virus, or to specific directed mutations. It is now generally agreed that the alteration is more constructively considered as a restricted transfer of genetic material to the cell, rather than as an induced mutation and the reviewer has proposed *transduction* for such processes, to avoid the diverse connotations of transformation (10, 58).

So long as the transductions influenced only a single specialized trait, the capsular antigen, an infective plasmid furnished a plausible interpretation, especially by analogy with latent virus. However, many transducible characters have been studied in several bacteria (pneumococcus, 10, 58, 81; *Hemophilus influenzae*, 1; *Salmonella typhimurium*, 222). In *Salmonella* these thirty or more traits include biochemical syntheses (nutritional requirements), fermentations of sugars, resistance to antibiotics, and flagellar antigens: Most or all of the heredity of these bacteria can evidently be transduced, but only one unit at a time. Thus, transduction is functionally, and perhaps phylogenetically, a special form of sexuality, which follows a more familiar, coordinated pattern in related bacteria, i.e. *Escherichia coli* (103). The possibility of transduction of genetic factors in higher organisms has been suggested by studies on the transmission of tumors by presumably cell-free extracts of chromatin or mitochondria (225, 227). The likelihood of confusion or identification of the transducing fragments with tumor viruses is obvious.

The genotype is a priori unlikely to consist of a large number of mutually unorganized autonomous units (102, 131, 219; also see discussions of 58, 81). Without incessant reduplication of each unit, the system could not long remain qualitatively intact. In a complex organism, some mechanism must insure a meristic separation of each hereditary unit at every fission, but it is difficult to conceive of such an operation for an inordinate number of independent units. For the same reason that amitosis is suspect in higher forms, we postulate that most of the genetic material is coordinated in a definite structure. The only candidate whose availability is certain is the nucleus, in which desoxyribose nucleic acid (DNA) has been cytochemically demonstrated (21). Chemical and enzymatic studies have shown that transductive competence in the pneumococcus and influenza bacillus is closely associated with DNA. A single particle of DNA might escape unnoticed in the cytoplasm, but if every trait is referable to DNA, this must be located where it is visible, that is in the nucleus.

The apparent fragmentation of the genotype in transduction, whereby individual traits are separately transferred, thus need apply only to the transmission and not to the internal organization of the genes either in host or recipient. Whether agents of transduction are chromosome fragments or macromolecules containing DNA (10, 59, 103, 131) is entirely a matter of outlook. In either event, genetic material

is transferred from the fragmented structure of the host cell, and reorganized into the recipient. The characterization of this material as a plasmid rests squarely on the hiatus of our present genetic knowledge of the bacterial nucleus. Until this is much more thoroughly understood, infective transmission from cell to cell cannot be relied upon as a criterion of extranuclear residence.

The chemical composition of transducing agents has important implications, if they can be regarded as purified genes. The most active preparations from the pneumococcus have been refined with the methods of nucleic acid chemistry, and consist almost entirely of polymerized DNA with less than 1 per cent protein. Furthermore, these preparations are rapidly inactivated by desoxyribonucleo-depolymerase, so there can be little doubt that DNA plays an essential role. Whether protein has been completely excluded is a more difficult problem (127). The biological activity of the preparations is less impressive when stated in molecular than in millimicrogram units. There is ample room for an undetected active nucleoprotein contaminant floating in an excess of purified, viscous, polymeric, inactive DNA. Preparations of isolated chromosomes from spermatozoa contain only a few per cent of a residual structural protein. Hypothetically, the retention of activity in preparations fractionated to leave less than 1 per cent of this protein would not rule out its possible biological importance.

The most plausible conclusion from existing studies with the pneumococcus is that DNA is not only necessary but sufficient for transductive activity. However, plausibility must be fortified by rigor before generalizations as to the overriding role of DNA in genetic specificity can be acquiescently accepted. The biochemical studies to date appear to have been designed to conserve the native nucleic acid, and this might itself account for the trend of the experimental results.

The advantages of *Salmonella* for genetic studies are countered by the inaccessibility of the transductive agent for chemical analysis, for it is apparently associated with bacteriophage particles. The passive role of phage as a vehicle for the transmission of genetic material should not be confused with the active determination of certain traits by latent viruses, as reviewed below.

#### ENDOSYMBIOSIS

The plasmids of previous sections were first detected by genetic tests. After the rules of their transmission had been worked out, cytoplasmic particles were often identified with the plasmids, although the observed particles might often be equally well understood as secondary products of plasmid action. There are many hereditary endosymbioses which have been detected morphologically, and have not received the attention they merit from geneticists.  $\kappa$ , a plasmid in *Paramecium aurelia* represents one instance that eventually would have been discovered by the morphologist. Our account of  $\kappa$  (see section, *Cytoplasmic inheritance in Paramecium*) may therefore be resumed where its history approaches that of other endosymbionts.

The all-or-none transmission of  $\kappa$  in suitable conjugation experiments originally suggested its particulate nature. The first estimates of its numbers came from kinetic studies of its attenuation in *variety 2*, in which the killer trait had been observed to be unstable. When the  $\kappa$ -containing animals are grown with optimal nutrients, the cells evidently multiply faster than the  $\kappa$  particles, so that the latter become progressively diluted at each fission until some animals are entirely free of  $\kappa$ . It was shown kinetically that a single  $\kappa$  particle could maintain the killer trait (if the animals were then grown more slowly), and that the stable  $\kappa$  population consisted of  $10^2$ - $10^3$

particles per cell (149). Later, it was found that temperature shocks and x-ray treatments differentially inactivated the  $\kappa$ . Its unexpectedly large radiosensitive cross-section led to the microscopic search for and identification of  $\kappa$  as a Feulgen-positive particle about  $1 \mu$  in diameter (150). Concurrently, Sonneborn succeeded in the artificial infection of sensitive KK paramecia with  $\kappa$  from concentrated breis of killer animals (181). Killer cells can be chemically disinfected with nitrogen mustard and with chloromycetin (26, 182). Despite superficial references to rickettsia, the taxonomic position of  $\kappa$  remains in doubt, but not its assimilation as an endosymbiont (cf. 2, 5, 182).

Hereditary endosymbioses are probably more prevalent than many biologists are accustomed to believe. Buchner (28) has collected the literature on plant-animal symbioses in his magnificent book. Had he further emphasized the hereditary character of these associations, the present review would be superfluous. A complete revision of *Endosymbiose* (29) has been announced, but was not available in time for this writing. Most of the literature on symbiosis is morphologically oriented but new nutritional knowledge is likely to make the biochemical basis of endosymbiotic relationships far more meaningful. Too little emphasis has been placed on the occurrence and behavior of 'disinfected' or aposymbiotic individuals, and on the criterion of reinfection for the specificity and identity of the endosymbiotic microorganism.

Owing to the simplicity of their overt cell structure, cyanophyte (blue green algae) endosymbionts have repeatedly been interpreted as intracellular organelles, so that the 'syncyanoms' are especially instructive (142). They include such complexes as *Geosiphon* (a phycomycete + *Nostoc*) and *Gloeochaete* (an apoplastidic chlorophyte + a cyanophyte). But the most interesting associations occur when the cyanelle (Pascher's term for a cyanophytic endosymbiont) is regulated by the host. *Paulinella chromatophora* is a testaceous rhizopod which regularly carries two sausage-shaped blue chromatophores, so that Lackey (96) insisted they were cell organelles. Functionally they are, for this animal exhibits a holophytic nutrition, contrary to the voracious phagotrophy of related testaceans. The division of the rhizopod is accompanied by the segregation of the cyanelles, which later divide to restore the count of two in each daughter cell.

*Pellicina cyanea* is a flagellate (chryomonad or cryptomonad in its affinities) which harbors from one larger to six smaller cyanella. Rare asymmetric fissions yield colorless monads which produce oily reserves rather than starch like the *Pellicina* complex. Since free cyanophytes do not form starch either, the complex has achieved a new function. The syncyanom must be of some antiquity, as there is no way that the flagellate could have ingested the cyanella. Phagotrophic, amoeboid phases have been described for some primitive flagellates, and the ingestion of a free-living cyanophyte by an amoeboid ancestor presumably initiated the symbiosis, to replace, at least functionally, the initial plastid system.

Other types of algae are also involved in endosymbiotic complexes: A large part of the phytoplankton of the sea may be bound as zooxanthellae and zoochlorellae (221). Some of the symbiotic chlorellae may afford superior experimental material for further work. The association of a chlorophyte with a flatworm, *Convoluta roscoffensis*, is the subject of a classical account (91), but the hereditary association is marginal: The algae are transmitted in the egg capsule, rather than within the egg (as in many sponges and the familiar *Chlorhydra viridissima* (cf. also 69).

*Paramecium bursaria* is one of the best known zoochlorella complexes, and has

also been genetically studied in other aspects (82, 141, 151, 178). The symbiosis can be maintained indefinitely in the light as a holophyte, whereas other paramecia require complex media with still undefined constituents. In the dark, however, the chlorellae deteriorate and may subsequently be digested by the animal. Thus, aposymbiotic animals are fairly easy to secure, and can be maintained like other species. Free-living chlorophytes of various species were ingested but not stably established, but zoochlorellae expressed from green animals were readily incorporated. Following a series of negative reports, the isolation and culture of zoochlorellae has been described, opening the door to studies of genetic variation of both components of the complex (74, 109, 178). The chlorellae have also been removed with x-rays (207, compare  $\kappa$ ). Symbionts of certain clones are toxic to others; it is not known whether this is related to the lethal mating reaction observed in certain combinations, or to the  $\kappa$  effect in *P. aurelia*.

In several chlorella-symbioses, particularly *Pelaiina* and *P. bursaria*, the chromatophores mediate a phototactic response, probably an indirect effect through CO<sub>2</sub> utilization (142, 151).

#### INSECT SYMBIOSES

Symbioses of microorganisms with insects are very prevalent. Many are intracellular and hereditary; others have equally effective techniques for persistent occurrence. They have been most thoroughly examined by morphological techniques, and very little physiological detail is known (28, 98, 187, 188, 208), although a nutritional function is almost self evident. A special organ, the mycetome or pseudo-vitelus, harbors a high concentration of the microsymbionts in many insects; in others, they occur in certain areas of the gut wall. The origin of the mycetome in *Pseudococcus* (mealybug) from the polar bodies disputes its homology with endodermal symbiont-carrying cells in other insects (cf. 171, 188).

The penetration of the symbionts into the egg occurs at different stages in different organisms. This usually occurs early in the development of the oocyte; a nutritional relationship between the ovary and the mycetome has been suspected by several investigators (6, 71, 138). In many beetles, however, the egg surfaces are contaminated as they pass through the ovipositor, and the larvae or nymphs are infected only after they feed on the egg shells. (The extracellular symbiotic protozoa of termites are similarly transferred by fecal ingestion, 82, 92.) In bostrychid beetles, seminal transfer has been recorded. In *Glossina* and pupiparids, the milk-gland secretions are implicated. Many of these processes have a counterpart in the transmission of agents primarily studied as plasmagenes.

The taxonomic identification of the microsymbionts awaits unequivocal proof of their cultivation *in vitro* (or *in ovo*), rarely accomplished. The rickettsia are the most notorious symbionts; as many authors have pointed out, their inheritance in arthropods contrasts with their virulence in mammalian hosts (187, 188). The bacteria-like symbionts have not been certainly cultivated (68, 174); if a preliminary report that they lack Feulgen-positive nuclei is substantiated and generalized, it is not likely that they can be identified with free-living microorganisms (97). Yeast-like forms have been cultured from several beetles, and probably do correspond to their symbionts (132, 140).

Koch's postulates provide reasonable criteria that should be met for any organism that can be cultured *in vitro*, and of which the insect can be at least temporarily disinfected. Even without *in vitro* culture, reimplantation and cross-implantation would provide important information, but this avenue has been little exploited.

Both biological and physiological studies of the symbioses are facilitated by techniques for aposymbiosis: the disinfection of the insect. This has been accomplished in several ways; surface sterilization of contaminated eggs, heat treatments, surgical or centrifugal removal of the mycetome, and, more recently, antibiotic therapy (6, 25, 27, 28, 71, 95, 134, 140, 187). Nutritional functions of the symbionts have been clearly shown in several insects. In the reduviid (assassin bug) *Rhodnius prolixus*, aposymbiotic nymphs failed to moult after the fourth instar, despite repeated blood feedings. The suspended nymphs resumed normal development when experimentally re-infected with cultures of the symbiont, *Streptomyces rhodnii*. Attempts to substitute other actinomycetes were not reported (25).

The flour beetles *Stegobium (Sitodrepa) paniceum* and *Lasioderma serricornis* carry distinct yeast-like forms, *Saccharomyces anobii* Buchner. Heterologous and homologous cultures were successfully re-implanted, but specific adaptation to the homologous host was indicated with respect to the availability of B vitamins synthesized by the yeasts (94, 140).

Most aposymbiotic insects are developmentally or reproductively defective (71, 187, 188, 208), but the granary beetle *Oryzaephilus surinamensis* is exceptional (95). It may be necessary to conduct careful nutritional experiments with aseptic precautions to define the nutritional functions of some symbionts.

Carter's account of the Hawaiian pineapple mealybug, *Pseudococcus brevipes*, is the closest rapprochement of these studies to genetics. The insect secretes a wilting toxin which is associated with a green-spotting of the pineapple leaves. When type colonies were transferred to panicum grass, the next and succeeding generations of mealybugs lost the green-spotting potentiality. This loss was associated with the disappearance of a rod-shaped symbiont from the mycetome, whereas other forms persisted (36). The type culture is gray and obligately bisexual; the variant is pink and parthenogenetic, only females (hermaphrodites?) developing (85).

The multiplication and hereditary transmission of a phytopathogenic virus in its insect vector has been reported for the rice stunt, clover club leaf, wound tumor and aster yellows viruses (18, 65). The first two of these have been maintained in the insect vectors for many generations, where they are inherited matroclinously. Careful cultural techniques, and the use of nutrient plants immune to the virus, have left no doubt of the active proliferation of the 'plant virus' in the insect. Inheritance of the wound tumor virus is irregular, and has not yet been demonstrated for the aster yellows, but the viruses can be propagated by serial inoculation of the insect hosts. Genetic factors apparently play some role in the maintenance of the viruses (18; cf. 191). Like so many plasmids, the viruses can often be removed by differential heat treatment of the infected insects.

These viruses have had no detectable effect on their animal hosts, and might therefore be readily overlooked, equally by insect pathologist or geneticist. Such infections may, however, have very subtle effects not discernible in ordinary cultures. An infective but noncontagious hereditary agent,  $\sigma$ , has an increased susceptibility to narcosis by CO<sub>2</sub> as its sole effect on *Drosophila melanogaster* (105). The plasmid is inherited matroclinously, independently of any chromosome markers. Some seminal transmission is also found, probably via polyspermic fertilizations.  $\sigma$  is also transmissible by artificial inoculations of hemolymph or by organ transplantations from infected donors. However, patroclinous transmission occurs only when the male was already infected at the egg stage, in accord with the early separation of the germ line in *Drosophila*.

$\sigma$ -infected, CO<sub>2</sub>-sensitive flies may be somatically or germinally cured by heat

shocks of the larvae. Incomplete or delayed treatments give only temporary or somatic cures. The distribution of the plasmid and its latency would seem to depend on the stage at which the organism is infected: the developing egg (in an infected mother), at fertilization (from an infected father), or later in development (by inoculation).

A mutant  $\sigma$  has been described which is less readily transferred via the male germ line. In addition, relapses following inadequate heat treatment of the larvae give infections resistant to a second heat shock, perhaps by a modification of the fly, or of the  $\sigma$  by selection of a heat-resistant mutant.

Filtration experiments have indicated a particle size of about  $0.1 \mu$ , but  $\sigma$  has not been further characterized. It has been transferred to some other *Drosophila* species by inoculation, but not to other *Diptera*, nor has it been detected in them. Because it is not contagious in ordinary culture,  $\sigma$  has been described as a genoid, intrinsic to the organism. However, infection from food or ectoparasites has not been disqualified. Until  $\sigma$  recurs, the problem of its unique origin will not be solved.

#### OTHER LATENT VIRUSES, IN PLANTS AND MAMMALS

The presence of a virus in an infected organism may be hidden in a number of ways (5, 11, 34, 48). In the most subtle form, the virus cannot be detected as an infectious agent, and infection is inferred only from the previous or subsequent history of the organism. This category would include the masked Shope papilloma virus in the domestic rabbit, swine influenza in the lungworm vector, and lysogenic bacterial cells as discussed below. In a second category, free virus is present, but pathological effects may occur if at all only with very heavy infestation, or under special environmental conditions, as in lysogenic bacterial cultures, the mouse hepatitis virus, herpes simplex in man, and most of the latent X viruses of plants. Bacterial cells are distinguished from cultures to emphasize the probability that individual infected cells may be damaged by a latent virus, without detectable effects on the whole organism.

Pollen and seed transmission of infective viruses is exceptional in plants but occurs sporadically in legumes. The special property that isolates the seed from virus rampant in somatic tissues "remains one of the most interesting unsolved problems of virus diseases" (11). The agents of the hereditary plastid variegations, on the other hand, have not been artificially transmitted, although a persistent experimental attack is perhaps still needed. The mode of transmission of these agents constitutes a plausible but only empirical basis for classification as viruses, 'proviruses,' or plasmagones (45).

Several mammalian viruses have a quasi-hereditary transmission, including murine hepatitis (72) and lymphocytic choriomeningitis (196), the exact route being unknown. One etiological factor in the development of mammary tumors in mice, previously characterized as a maternal influence, has been shown to be transmitted primarily by the milk. The agent occurs throughout the tissues of infected animals, and can also be transmitted by artificial inoculation and possibly via the semen and *in utero*. Its pathological consequences and maintenance are under chromogenic control. It has been propagated in chick eggs and in male mice, in which it remains asymptomatic in the absence of hormonal stimulation. The viral and the genetic properties of the milk agent have both been emphasized (4, 108, 130). The flourishing interest in latent viruses along lines similar to those of this paper is exemplified by a symposium (224) and review (226) which appeared while this was in press.



As a converse to the modification of host cells by viruses, there are several examples of virus alteration in different hosts. Host adaptation is probably most often a consequence of spontaneous mutation and selection (33, 34, 112), but phenotypic modification of a virus has also been considered (78) as well as genetic recombination of the invading virus with previous residents of the cytoplasm (35, 79, 164).

#### LYSOGENIC BACTERIA

Stable symbiotic associations of bacteria with viruses (bacteriophages) occur very frequently. Because the symbiosis is detected by the lysis of a sensitive indicator strain, the association has been called *lysogenicity*. The lysis of an indicator reflects the production of free virus only by few of the bacteria in a lysogenic culture. Most of the cells perpetuate the potentiality of producing virus, although the virus itself is rarely detectable in them. For this reason, such cells are considered as infected with a *provirus*, a perpetuating, but immature and nonlytic agent. (The same expression has been used in other meanings. As used here, and by several authors, 24, 48, 117, *provirus* is a formal designation for the nonlytic stage of development of the symbiont; its application should not conceal our ignorance of the differences, if any, between the hypothetical provirus and free virus particles.)

Lysogenicity has been used as an argument for the endogenous origin of bacteriophage (70, 217). However, since foreign viruses can enter into the same symbiotic relationship that is displayed by bacteria lysogenic when first isolated, the argument is inconclusive. On the other hand, symbiotic viruses which may be difficult to detect as such occasionally mutate to virulent forms, simulating the *de novo* production of phage from a normal bacterial culture (23, 100). Neither of these observations contradicts the intracellular-parasite theory of phage, as enunciated by its discoverer (33, 49), but this in turn does not rule out hereditary functions of the symbiont.

Little detail is known of the initiation of the lysogenic symbiosis. For technical reasons, recent phage studies have concentrated on atypically virulent strains, particularly the 'T phages' acting on *E. coli* B (46-48). Infection of a bacterial cell with a single T phage particle promptly interrupts normal biosynthetic activity, which appears to be almost completely diverted to the demands of the virus. After a dark period of several minutes, during which the infecting phage is undemonstrable, there is an interval of rapid increase of virus, as detected by chemical analysis or artificial rupture of the bacteria (41, 52). The growth period culminates in bacterial lysis, and the release of some dozens or hundreds of free virus particles.

Certain phages, designated as temperate for particular hosts, may follow a different course alternatively to the lytic pathway. The stages intervening between infection and symbiosis are obscure but a bacterial clone develops in which a dark period is indefinitely prolonged while normal growth proceeds. Later, descendants of the infected parent may sporadically resume a lytic pathway and release free phage. Unless this phage reaches a sensitive indicator strain, it is not detectable.

The proportion of bacteria which follow the alternative pathways of lysis (*LP*) or symbiosis (*SP*) varies with the hosts and viruses involved, and with the conditions of infection. In some staphylococci, *LP* predominates (165, 209); in enteric bacteria, *SP* may predominate to the extent that no overt lysis is observed at all (205). In *Salmonella typhimurium*, high multiplicities of infection appear to favor *SP*, suggesting a heterogenicity of the free virus population: hypothetical temperate variants initiate lysogenicity and protect against lysis (24). The hy-

pothesis of a reversible genetic differentiation between temperate and lytic free virus should not be confused with the verified occurrences of stable, lytic variants from temperate phages. The hypothesis has not received independent support but makes a useful framework for further discussion. Multiple or iterated infection per se affects development of lytic phage (51, 63) and might be directly involved in the prolongation of the dark period that culminates in lysogenicity. Alternatively, the LP/SP decision may rest on differentiation of the bacteria, for which genetic studies offer some support.

The lysogenic symbiosis is, *prima facie*, plausibly regarded as the carriage of provirus in the cytoplasm. To explain resistance to lytic attack by the provirus, to free virus, and sometimes to related viruses, one may postulate the same kind of interference as has been demonstrated in lytic systems. This simple picture has not, however, been supported by crossing experiments with *Escherichia coli* K-12, which point to a single chromogene as the major determinant of the sensitive, lysogenic and immune states (100). It is not settled whether lysogenicity results from the selection of spontaneous mutants when sensitive cells are infected with the virus,  $\lambda$  or whether  $\lambda$  more directly induces the alteration of the chromogene. If the latter is supported by as high an efficiency of formation of lysogenic from sensitive cells as appears at first sight, a chromosomal localization of the  $\lambda$  provirus will have to be given serious consideration. A special relationship between the nucleus and some viruses may also be inferred from cytological and cytochemical analysis of phage infected bacteria, and from the attachment to certain *Salmonella* phages of genetically active material of the host cell (41, 113, 133, 222).

The most prominent phenotypic effect of lysogenicity is the resistance acquired against the symbiotic phage, and often against other intemperate phages. The pattern of resistance to a standardized series of phages, the lysotype (44, 137), serves to characterize individual strains of several bacterial pathogens for which epidemiological tracers are needed. In staphylococci (165, 209), *Salmonella typhi* Vi-types (3), *Salmonella paratyphi* B (137) and other *Salmonellas* (210), the lysotype is determined to a large extent by the carriage of determinant phages. Such phages are as a rule closely related to the typing phages themselves. In *Salmonella typhi*, the typing phages are specifically adapted by growth on standard Vi strains, and will lyse only cells carrying this antigen. The determinant, symbiotic phages show no Vi-specificity in their own host range. The mechanism of adaptation of the typing phages has not been closely studied, but may be either a selection of genotypic variants, or a phenotypic modification of the typing phage imposed by the host cell (or its proviral symbionts if these can be distinguished from the host).

Other effects of phage plasmids have been less extensively studied. Toxicogenicity is induced in avirulent strains of the diphtheria bacillus by infection with temperate phages (62). The El-Tor vibrio, distinguished by its hemolytic activity, is claimed to be a lysogenic derivative of *Vibrio cholerae*; many bacteria release hemolysins when lysed by phage (53, 167). Other 'transformations' or filtrate effects should be re-examined for the simple participation of phage (e.g. 64, reviewed 101, pp. 181-184).

The long mooted question whether phage was continuously secreted by lysogenic bacteria has been conclusively answered. In *Bacillus megaterium*, the appearance of free phage is always correlated with the lysis of one or more cells, under microscopic observation (117). Similarly, free phage in replicate cultures of *E. coli*, strain Lisbonne-Carrère, is distributed in discontinuous bursts. Bacteria may be lysogenic

for two or more phages, and still support the *LP* growth of an additional virus. In the experiments so far reported, individual bursts liberate one phage only (16, 201).

The resumption of *LP* occurs sporadically, but is also under experimental control. Some lysogenic strains of *B. megaterium* are evidently poised at a precarious balance, and exposure to small doses of ultraviolet light, x-rays, or reducing agents will induce massive lysis of the culture. Ultraviolet light will also induce *LP* in some, but not all, lysogenic associations of *Escherichia coli*. Consequently, infection often leads to sensitization to ultraviolet light, if the cells are tested under appropriate conditions (118). An antibiotic (colicin) producing strain of *E. coli* responds to UV in the same way; whether the colicin is itself a modified phage, or whether another undetected phage is concerned is uncertain (61, 86).

No straightforward method is yet available for disinfecting lysogenic bacteria (22, 32, 165), although sporadic successes have been reported (39, 54, 195). This may simply attest to the close integration of host and plasmid. Without such a technique, or an indefinite range of potential indicators, it is impossible to assert that any bacterial culture is free from the potentiality of producing a virus. In fact, in certain groups of bacteria the incidence of proven lysogenic isolates approaches one hundred per cent (23, 32, 60, 70, 124, 136, 169, 222). Conversely, lysogenicity probably constitutes the principal reservoir of bacteriophage in nature (33).

#### HETEROKARYOSIS, HYPERPARASITISM AND SEXUALITY IN THE FUNGI

The problem of delimiting the normal territory of the cell from included plasmids is magnified when the components are of equal stature. Coenocytosis occurs only in restricted tissues or phases in higher plants and animals (e.g. the insect blastoderm), but is a regular feature in mycelial and plasmodial microorganisms (many fungi and some algae and protozoa). So long as all of the nuclei of a coenocyte are genetically identical (homokaryotic), no special problem arises. However, heterokaryosis may arise by mutation, cytoplasmic fusions, or special sequelae of sporogenesis which bring diverse meiotic products together (161). The genetic and evolutionary consequences of heterokaryosis have been examined most closely in ascomycetous fungi (146, 147). The implications of syncytia as exceptions to the cell theory have been discussed recently (223).

In the heterokaryon, several nuclei operate in a common cytoplasm undivided by cell boundaries. The problem of delimiting the plasmid from the normal territory of the cell is exaggerated here, where we must cope with reactants of equal stature, the diverse nuclei. Ultraviolet light induces lethal mutations, probably chromosomal deletions, distributed among the component nuclei of heterokaryotic conidia of *Neurospora*. Homokaryotic segregates are therefore inviable, but different nuclei sustain each other to give a stabilized or balanced heterokaryon, comparable to balanced heterozygotes in *Drosophila*, or more particularly to obligate symbioses (7). The substantial basis of the interaction is not precisely known for the lethal mutations. However, nutritionally dependent (auxo-heterotrophic) mutants interact in a similar way to give auxo-autotrophic heterokaryons (14). The auxoheterotrophic mutation is a relative lethal, for it will survive only if its required growth factor is supplied to it, whereas the autotroph synthesizes the growth factor for itself. The number, location and kind of steps that lead from genetic determinant to growth factor product are not known. The activities of the nuclei in a heterokaryon might be pooled at any intermediate extranuclear level, be it growth factors themselves, biosynthetic enzymes, or less accessible, gene-controlled regulatory mechanisms.

Syntrophic interactions imply that the effective field of each nucleus is the entire cytoplasm, so that the genotype of the entire coenocyte is a summary average of the activities of the component nuclei.

The composition of heterokaryotic populations raises a question of local individuality of the nuclear field (146). Unlike simple heterozygotes, in which gene dosages are restricted to simple integral ratios, the proportions of a pair of alleles in a heterokaryon may vary continuously. But how are the ratios determined? If the individuality of the nucleus is submerged in the composite effects on the entire coenocyte, direct selective effects on particular nuclei are precluded. This concept is substantiated by many experiments in which auxo-heterotrophic mutant nuclei were indefinitely propagated in association with wild type in heterokaryons grown on minimal medium. Some exceptions must, however, be noted in which, for example, leucine-independent nuclei were displaced by leucine-dependent, even on a minimal medium which failed to support further growth of the latter, so that self-extinction ensued (166). Phenotypic individuality of diverse nuclei in a heterokaryon may therefore find expression in the determination of the nuclear ratios, of which closer study is needed.

Another field of action is the chromosome, as shown by position effects (43, 104). Several examples have been found of chromogenes which do not interact in heterokaryons, but must be in juxtaposition on the same chromosome. A further possibility has not yet been verified: a reaction limited by the nuclear membrane. Experimental material for it is available in those fungi where heterozygous diploid mycelia can be compared with heterokaryons (101, 163).

The evolutionary place of heterokaryosis has been evaluated with respect to sex and to parasitism (14). The heterokaryon permits the association of diverse genotypes, whose adaptivity is perhaps signified by the high incidence of heterokaryosis in natural isolates. The instability of the heterokaryon and the limited range of combinations it permits make it less productive than sexual recombination for potentially adaptive variation. It has also been suggested that heterokaryosis was an early phylogenetic step in the development of sexuality. However, the prevalence of recombination processes even in viruses suggests that sexuality preceded or coincided with the organization of the euploid nucleus.

Heterokaryotic associations are not confined to intraspecific combinations. In *Neurospora*, the interspecific heterokaryons tend to be somewhat unstable, and can be maintained only under selective pressure (146 and its discussion). Several cases of hyperparasitism have been described, particularly in the *Mucorales*, in which the parasite forms a heterokaryon with the host mycelium (31). It would be instructive by appropriate manipulation of the medium, or the use of artificial nutritional mutants, to test the mutualistic potentiality even of such parasitic heterokaryons.

#### SYNTROPHISM AND AUTOTROPHISM

Almost all green plants, and a few special microorganisms are autotrophic, that is are potentially self-sustaining in the absence of any organic nutrients. The remainder of the living world is dependent on other organisms, ultimately the green plants (93, 114, 170). Expressed otherwise, all heterotrophs are genically insufficient, and depend for their subsistence on the biosynthetically expressed genic functions of other organisms (13). Thus it is possible to formulate a graded series of symbioses as genic interactions, from the co-habitants of a single chromosome, through heterokaryons and plasmids, to extracellular ecological associations of variable stability

and specificity (III). Other writers have commented on levels of cooperation in societal rather than genetic terms, emphasizing the relationships of genetically similar units such as cells, and some have drawn holistic conclusions (80, 156, 204). Without furthering such extrapolations, our survey of symbioses should show that the delineation of organic units, be they genes, plasmids, cells, organisms, genomes or colonies is a tool of investigation and communication and not an absolute ideal. For different purposes the units may be different but there need be no contradiction between the Paulinella complex as a single chromatophoric organism (96) or as rhizopod plus cyanophyte (92).

#### SELF-REPRODUCTION AND SELF-DEPENDENCE

The concept of genetic autonomy or 'self-reproduction' has been implicit throughout this review, but has not been critically defined. When the capacity for reproduction is self-contained, as in a free-living organism, no serious question arises. But what criteria are applicable to smaller units of or in the larger complex, by which we can show that some are 'self-reproducing,' while others are reproduced by the organism as a whole? The problem is usually approached by abstracting the unit in question from the organism. If it is then no longer produced by the organism it is concluded to be self-reproducing. This has unfortunately been taken to mean that the unit is autotrophic, but the proof shows only self-dependence, not self-sufficiency. In this context, self-dependence describes a wide range of reactions, from simple autocatalytic reactions which may direct the chemical evolution of intracellular metabolites, to self-contained autotrophic organisms (2, 193, 219, 220). The position of the gene in this hierarchy is not established.

Experimentally, it may be very difficult to test a visible particle for self-dependence. Surgical excision is rarely applicable. Chemotherapy or biological incidence may indirectly remove a particle, but this does not preclude more remote effects. Only the most searching inquiry supporting the parallelism in distribution of a visible particle with that of plasmid effects can rigorously justify their identification with each other, as has been done with the chromogenes and chromosomes. When the plasmid can be purified by chemical or microbiological procedures its successful re-introduction will suffice as well.

The need for some circumspection concerning viruses and self-reproduction has been well illustrated by an 'alkaligenic virus' described in 1925 (110). Heavy inocula of *E. coli* on plates of a sugar-peptone-agar medium initially produce a uniform acid reaction by glycolysis, inhibiting further growth and enzymatic action. On one plate, alkaline reversal spread from its initial location, suggesting a growth process. When a piece of the agar was planted on a second plate, the alkaline reversal was propagated, suggesting the spread of an alkaligenic virus. In fact, reconstruction experiments (the reviewer, unpublished) show that the so-called virus is the  $\text{OH}^-$  ion. The local neutralization of accumulated acid permits the oxidation of the peptone, from which more alkali is released as ammonia. The simulated virus is a simple metabolic diversion. To insist on the self-reproduction of  $\text{OH}^-$  is a *reductio ad absurdum*.

To avoid such difficulties, a second criterion has been suggested: the capacity for propagable mutation. However, mutability may be merely a consequence of structural complexity, (as pointed out to the reviewer by C. C. Lindegren). All-or-none deviations of the components or rearrangements of their pattern are translated into mutations of the complex. The presence-absence concept as applied to mutation of the chromogenes has been discredited by the discovery of multiple allelic series,

which suggested innumerable discriminations of gene forms. More recently, however, a finer analysis of multiple alleles has revived the application of presence-absence and positional relationships to units distinct from the conventional single chromosome (73, 122, 185). A priori, the elementary units of genetic reduplication are likely to consist of only a few species, from which complex specificity is derived by structural organization. The units themselves are self dependently reproduced in the cell, but need not possess the mutability or individuality associated with the gene or organisms as a whole. On this basis, the elementary processes of reproduction may be as simple as the alkaligenic virus.

Oversimplified analogies between crystallization and over-all biological growth have been soundly criticized. But the selective deposition of homomorphic substituents and the perpetuation of specifically oriented faces and habits in the crystallization of nonelectrolytes do fire speculative reasoning on these elementary processes. Unfortunately, we do not know much more about the growth of crystals than of cells, but the immediate prospects are brighter (30).

The search for elementary processes of reproduction is urged on by speculations on the origin of life. Recent writing has tended to assume the accidental production of the first living protein molecule (13, 20, 139), discounting the improbabilities with the stipulation that life has evolved. An approach more consistent with general evolutionary theory would be to search for simpler units and processes (not necessarily proteinaceous) which bridge the gap between the organic and inorganic by innumerable steps each oriented by natural selection. As a corollary, we may question whether similar first steps toward abiogenesis do not occur frequently today, too ineffective for our recognition, and with little hope of successful evolution in competition with the far more efficient creatures of the living world. Transposed to the cell, this argument suggests that genetic autonomy may gradually arise in internal metabolites initially devoid of self-directive capacities as well as by mutation of pre-existent plasmids.

The complexity of genes, microorganisms, and visible plasmids makes them unpromising material for the problem of their reproduction. Therefore, we must look to simpler self-directive systems—perhaps including the ciliary antigens of protozoa, or priming factors in polysaccharide synthesis (77)—for suitable experimental models.

#### EVOLUTION OF THE CELL AND ORGANISM

Evidence from comparative cytophysiology and biochemistry supports the monophyletic origin of contemporary life (93, 114), but the lines are not always distinct. Speciation, especially in higher plants, has often been associated with hybridization of previously divergent groups. Endosymbiosis on the one hand represents a cooperation of distinct forms, on the other verges on hybridization (cf. 126).

We should not be too explicit in mistaking possibilities for certainties. Perhaps the disrepute attached to some of the ideas represented in this review follows from uncritical over-statements of them, such as the Famintzin-Merechowsky theory of the phylogeny of chloroplasts from cyanophytes (28, 126) or the identity of mitochondria with free-living bacteria (198). Similarly, the origin of viruses from normal constituents of the cell is likely to receive a more considered hearing from virologists and geneticists if the concept is not overburdened with dogmatic but unsupported generalizations as to the specific organelles from which they come. The generality of the subject demands imaginative speculation checked by the most cautious criticism.

This review has contrasted the various forms of plasmid: the hereditary parasites as against the functionally coordinated plasmagene, with the mutualistic endosymbionts somewhere between. It should not be assumed that the evolutionary pathways are unidirectional. The latent virus has become a normal constituent of the cell. If a pathogenic virus arises *de novo* tomorrow, shall we insist that the normal constituent from which it arose was not a latent virus yesterday? The question seems almost unanswerable, for the very reason that the plasmids may evolve. For example, it has been proposed that the paracrinkle virus of potatoes is an intrinsic derivative, since it is confined in nature, so far as we know, to the King Edward varietal clone, and was thought to be transmissible only by artificial grafts (45). Even on these premises, the argument is incomplete: The postulated normal precursor may have been an imported latent virus whose imprisonment in the clone was a subsequent adaptation (compare *Pelinaia supra*). This view has been strengthened by discoveries of fact: 1) the nonappearance of paracrinkle in the very rare King Edward hybrid seedlings, 2) successful mechanical transmission with the help of abrasives, and 3) completely symptomless infections of tomato by paracrinkle (12). The prevalence of virus latency in vegetatively propagated plants (potato, strawberry) is probably not accidental (11). The barriers to seed transmission of viruses would tend to preclude slow adaptations of plant and virus towards a symbiotic association. Where seed transmission of a latent virus has been accomplished, on the other hand, it is less likely to be recognized as it becomes more ubiquitous. Plasmon inhibitions (37), or genetic diseases simulating viroses (8, 159), might result from the recrudescence of inherited latent viruses when genotypic alteration upsets the symbiotic equilibrium.

In a search for experimental support of his 'viroplasm' theory, a predecessor to recent restatements of virus-plasmagene relationships, Johnson inoculated indicator plants with extracts of normal seedlings (87). New viruses were discovered but no conclusion as to their origin could be reached. The donor seedlings may have carried a seed-borne, symptomless virus. Whether such viruses are to be regarded as normal constituents, especially if ubiquitous within the species, is a question that strains our understanding of the normal.

This discussion illustrates how readily the properties of plasmagene may be imputed to viruses, and vice versa. The general criteria that have been used to decide the historical origin of particular plasmids are unverifiable, and such controversies have therefore tended to be sterile. It would be far more constructive to focus attention on plasmid functions which in terms of the whole organism may be now adaptive, later pathological. Present evidence points to the nucleus as the predominant, if not quite exclusive, seat of hereditary factors in most organisms. Whether new techniques will alter this generalization has still to be seen. However, we learn less, not more, if we ignore extranuclear agents as hereditary factors because they may also simulate symbionts or parasites; such behavior is important but incidental to their genetic functions.

The cell or the organism is not readily delimited in the presence of plasmids whose coordination may grade from the plasmagene to frank parasites. Many geneticists have pointed out further that the gene has displaced the cell as an ultimate unit of life (131, 219). How then shall we choose the boundaries of the gene-complex that constitutes an individual organism? If hierarchical definitions are to serve the scientist, rather than the scientist serve an Aristotelian category, different uses should dictate different usages. The geneticist may well choose that entity whose reproduc-

tion is unified and hence functions as an individual in evolution by natural selection (102). The microbiologist will focus his interest on the smallest units he can separate and cultivate in controlled experiments, in test tubes, eggs, bacteria or experimental animals. Genetics, symbiotology and virology have a common meeting place within the cell. There is much to be gained by any communication between them which leads to the diffusion of their methodologies and the obliteration of semantic barriers.

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