THE CYTOPLASM IN HEREDITY *

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I

DURING the past forty years many investigations have demonstrated that the cytoplasm has genetic functions. Correns (1909) and Baur (1909) led the way with their studies on variegation in higher plants. After observing cytoplasmic inheritance of a number of traits in mosses, von Wettstein (1927) set forth the concept of the plasmon, a system of genetic determiners in the cytoplasm. Plasmon-controlled traits have subsequently been reported in many other plants by a number of geneticists, foremost among whom are Michaelis and his co-workers on *Epilobium* (see P. and G. Michaelis, 1948, for literature). Comparable observations on higher animals are less common. L'Héritier (1948) and his colleagues have demonstrated cytoplasmic inheritance of sensitivity to CO₂ in *Drosophila*. Pigmentation in spotted guinea-pigs is interpreted by Billingham and Medawar (1948) to depend upon a cytoplasmic genetic particle. This spotting may be regarded as a manifestation of cytoplasmic inheritance on the cellular level. It is the commonest type of cytoplasmic inheritance in higher animals, probably occurring with regularity during their ontogeny, for different cell types taken from the same soma may maintain their diversities through countless cell-generations in tissue culture. Comparable cytoplasmic inheritance on the cellular level appears also in unicellular organisms, both in the form of hereditary variations within a clone and otherwise, as in *Paramecium* (Sonneborn, 1947a) and yeast (Ephrussi, L'Héritier and Hottinguer, 1949).

Cytoplasmic inheritance among both plants and animals, and in multicellular as well as unicellular organisms, is thus not an hypothesis, but a fact—one of the capital facts of biology. Hypothesis and opinion enter only when this fact is interpreted, when the physical bases of cytoplasmic inheritance and the mechanisms by which they operate are discussed. Even on the questions of physical basis and mechanism, there are a number of solidly established facts in one group of examples of cytoplasmic inheritance, but not in another group. To distinguish between the more and the less understood types of cytoplasmic

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inheritance, I shall separate my accounts and discussions of them into different sections of this paper. Section II includes the relatively well-understood type, section III the poorly understood type.

As will appear, this plan emphasises the existence of fundamental differences between different examples of cytoplasmic inheritance. These differences are not immediately apparent. Indeed, the different examples may at first seem to be similar in principle. I have learned this the hard way. In one of my first reports (Sonneborn, 1943) on cytoplasmic inheritance, it was pointed out that all nine known characters in several varieties of *Paramecium aurelia* showed superficially similar cytoplasmic inheritance and it was assumed the same sort of physical basis and mechanism was operating in all of them. The nine characters were of three kinds: (1) the production of antibiotic agents, *i.e.* the so-called "killer" traits; (2) serotypes or antigenic types; and (3) sex-like differences or mating types. One of the killer traits was analysed in detail in order to provide a model applicable to the other traits in *Paramecium* and possibly also to the traits in other organisms. This model does apply (so far as present information goes) to all of the killer traits and to many examples in other organisms; but it failed to apply to the next kind of trait examined in *Paramecium*, the antigenic types. Apparently more than one model is needed to account for the various examples of cytoplasmic inheritance.

Our experiences with *Paramecium* indicate that the fundamental distinction among these diverse examples lies in the source of the specificity of the cytoplasmic basis of heredity. In one group, the nuclear genes are the source of specificity; in another group, the cytoplasm itself controls its own specificity. The examples of the latter group seem to conform to a common model, the one developed in our study of the killer trait. In the following section an attempt is made to present and illustrate that common model, at the same time pointing out the gaps in knowledge, the uncertain aspects of the interpretation, and the lines along which may come further advances in knowledge and understanding.

II

(a) Self-duplication and mutability

The examples of cytoplasmic inheritance to be discussed in this section should be confined strictly to those in which the cytoplasmic basis of heredity is mutable, and therefore also self-reproducing. On these points the evidence is convincing and complete in but few cases. That a single model structure or particle is not only essential but sufficient, in the proper cellular environment, for the formation of more has been shown both for chloroplasts (Ternetz, 1912) and for kappa, the basis of the killer trait in *Paramecium* (Preer, 1948a). Those structures are clearly self-reproducing, as are many others to which we shall turn presently; but mutability is known only for the plastids (see review by Rhoades, 1946), for kappa (Dippell, 1948,

and unpublished), and for sigma, the basis of sensitivity to CO_2 in *Drosophila* (Goldstein, 1949). The work on mutability will be illustrated by the study of kappa, but as the killer trait it controls will be referred to repeatedly in the ensuing discussion, I shall first digress enough to provide the necessary background (see Sonneborn, 1943, 1945, 1946, 1947b).

Killer strains of paramecia liberate into their culture fluid a poison, paramecin, to which they are resistant, but which kills in a characteristic way paramecia of other strains, known as "sensitives." These sensitives can be mated to the killers, however, because sensitives are unaffected by paramecin during conjugation. The nuclear processes of conjugation are such that the two mates of a pair acquire identical genotypes. They then separate and each produces a culture by repeated fissions. In spite of their identity in genotype (or at least in *nuclear* genotype), the cultures derived from the two mates of such a conjugant pair are diverse: one is a killer, the other is a sensitive culture. From these F_1 cultures, the F_2 obtained by selfing shows no segregation: all F_2 cultures derived by selfing among the F_1 killers are killers; all F_2 cultures derived by selfing among the F_1 sensitives are sensitives. Hence, the difference between these killers and sensitives is due, not to a genic difference, but to a cytoplasmic difference.

This conclusion is confirmed by exceptional conjugations in which cytoplasm visibly flows from mate to mate across a connecting cytoplasmic bridge. After receiving cytoplasm from its killer mate, the sensitive conjugant produces a culture of killers. As before, no segregation occurs in the F_2 . From the fact that killers can transmit the physical basis of the killer trait to sensitives across a cytoplasmic bridge, it was inferred that the killers possess a cytoplasmic genetic factor determining the killer trait. This is the factor known as kappa.

After some years of study of the properties of kappa by indirect methods, Preer (1948b and in press) was able to demonstrate that it is a microscopically visible particle. Fig. 2 shows the presence of a large number of kappa particles in the cytoplasm of a killer; Fig. 1 shows their absence from a sensitive. Killers can be irreversibly transformed into hereditary sensitives by freeing them of kappa through exposure to high temperatures (Sonneborn, 1946), X-rays (Preer, 1948b), or nitrogen mustard (Geckler, 1949) and in other ways (Preer, 1946). Sensitives can be transformed back to hereditary killers by exposing them to concentrated suspensions of the broken-up bodies of killers (Sonneborn, 1948a). In this way, the sensitives acquire some of the kappa that had been liberated from the disintegrated killer animals. Kappa is thus a self-multiplying cytoplasmic particle which is formed only when some is already present.

The sensitives thus far described have the same genes as the killers and differ from them only in lacking kappa. There is, however, another kind of sensitive that not only lacks kappa, but carries different

genes from the killers. This kind of sensitive strain cannot be converted into a killer by exposing it to kappa released from the macerated bodies of killers. Further, when it is crossed to a killer, the F_2 generation derived by selfing among the killer F_1 clones, shows segregation of killers and sensitives in proportions expected for a single gene difference. Only when dominant gene K is present can kappa be maintained; in homozygotes for recessive k kappa cannot be maintained and any carried over from killers is quickly and irreversibly lost. Other loci also seem to be involved, but less strikingly, in the maintenance of kappa (Sonneborn, 1947b).

With this background, we may now turn to the study of mutability of kappa. Dippell (1948 and 1950) collected a number of mutations of the killer trait in stocks of variety 4 of *P. aurelia*. All the mutations were discovered in laboratory cultures of killers. Some of the mutants produce very little of the killing substance, paramycin, but remain resistant to its action. Other mutants produce a different kind of paramycin that kills sensitives with visibly different pre-death changes. When the type of killing changes, the pattern of resistance also changes, each kind of killer being resistant only to paramycin of the same type that it produces. Some mutants produce both kinds of paramycin and are resistant to both. Thorough breeding analysis demonstrated that these mutations involve no change in any gene affecting kappa or the killer trait: with respect to such genes, the original killer and its derived mutants are identical. In every case, the change could be traced to a change in kappa. This was most strikingly shown in the case of the mutants that manifested two kinds of killing. Dippell showed that these carried two kinds of kappa. By diluting kappa down to a single particle per cell and then letting it accumulate again, she was able to get "pure cultures" of the two kinds of kappa in different paramecia and their progeny. Thus kappa can mutate and the diverse mutant kappas can be maintained and multiplied in cells with the original genome.

Dippell's analysis proves that the specificity of kappa is inherent in kappa itself. With this the nuclear genes have nothing whatever to do: diverse kinds of kappa not only multiply true to type in diverse cells with the same genotype, but they even multiply together and maintain their specific diversities in one and the same cell and all of its cell progeny. The same sort of evidence for mutability of chloroplasts and sigma proves that they also are autotrophic and mutable, that their specificities are determined by themselves, not by the nuclear genes.

While the full demonstration of both self-duplication and mutability has been given only for the three examples already mentioned, a large number of others probably belong in the same category. There is some evidence for both self-duplication and mutability of the plasmon in *Epilobium* (Michaelis, 1948) and for a number of so-called "viruses" that resemble genetic factors in their normal transmission

only by inheritance, not by infection (see Darlington, 1944 ; Haddow, 1944 ; Sonneborn, 1949). A number of cellular structures, although not known to be mutable, are visibly self-duplicating. This has been emphasised by Lwoff (1949) for the cinetosome of protozoa, the granule that lies at the base of each cilium and flagellum ; by Darlington (1949a) for the centrioles ; and by Sonneborn (1949) for the bacteroids and Rickettsias of insects, for mitochondria, for the basis of male sterility in a number of plants, and possibly for the microsomes. To this list should be added the anlage of the gullet of *Paramecium* (Sonneborn, 1947a, p. 273), and perhaps the physical basis of the "small-colony" mutant in yeast (Ephrussi, L'Héritier and Hottinguer, 1949). Essential structures of a cell, such as centrioles and cinetosomes, may not be capable of surviving a mutation with effects gross enough to be otherwise detected. Yet the visible self-duplication of these cytoplasmic structures shows they can scarcely lack genetic significance. Without evidence as to their mutability, however, the question of whether they or the nuclear genes control their specificities cannot be answered.

(b) Size and organisation

Attempts have been made (Darlington, 1944) to classify these cytoplasmic genetic factors on the basis of their size and structural complexity and to attribute different significances to the different classes. The chloroplast is certainly one of the largest, but it arises from a much smaller plastid which is of the same order of size as many other cytoplasmic genetic particles : mitochondria, centrioles, cinetosomes and kappa. Somewhat smaller are the microsomes which may also belong in the category of genetic particles. I have heard that sigma of *Drosophila* is believed to be still smaller. It seems that self-duplicating cytoplasmic structures may grade down from the giant chloroplasts to macromolecules not much, if at all, larger than the nuclear gene. If the size series is as continuous as now seems likely, the usefulness of interpretations based on different sizes may be questioned.

Size can hardly be considered apart from organisation and development. Because chloroplasts have the genic properties of duplication and mutability and because they develop from much smaller plastids, as giant salivary gland chromosomes were believed to develop from ordinary chromosomes, Imai (1937) postulated the plastogene as a genetic determinant within the chloroplast. He went even further and assumed that each chloroplast contains a single plastogene, because of the lack of evidence for plastids with mixed constitution.

The question thus arises as to whether there are grounds for believing that other visible self-duplicating bodies consist of smaller genetic units, as is presumed in the case of the chloroplast.

There are indeed some reasons for suspecting that kappa particles

may consist of smaller genetic units. Several lines of evidence suggest that some kappa mutants may arise in pairs by a breaking of the original kappa particle into two smaller particles. Three of Dippell's five killer mutants carried two different kinds of kappa when first detected. In at least two of these three mutants, both kinds of kappa differed in some way from the original. Since killer mutants have rarely been found, the existence of two mutant kappas in even one of the few killer mutants could hardly be a mere coincidence, unless there were some unknown reason why double mutants are much more likely to be detected than single mutants. It is further remarkable and suggestive that at least some of the mutant killers can carry about twice as many kappa particles as the original killer. Finally, Preer (in press) records that the kappa particles in killer stock *G* are about 0.4 micron long, while those in the derived mutant *G m1* are only about 0.2 to 0.3 micron long. In other stocks (not mutants of *G*), the size of kappa goes up to 0.8 micron.

These observations, taken together, suggest that kappa exists in multiples of some basic size unit. As to how small the basic unit may be, or how many such units are present in the smallest visible kappa particle, there is little on which to hazard a guess. Preer's report (1948*b*) that a single hit curve describes the X-ray inactivation of stock *G* kappa might be considered as weighing against the multiple unit organisation of kappa, but his later work (in press) raises doubt as to whether the inactivation of kappa is after all a single hit affair.

To the indications of a finer genetic structure in chloroplasts and kappa, may be added comparable indications for mitochondria and centrioles. As is well known, mitochondria have been reported to arise *de novo* (Forenbacher, 1911, and later observers), to increase and decrease in size from and to the limits of microscopic visibility (Lewitzky, 1924; Kassmann, 1926), and to divide (Horning, 1926, and others). Supernumerary centrioles also appear to arise *de novo* under some conditions and then to divide (Wilson, 1901, and later observers). These evidences of *de novo* origin of self-duplicating structures suggest that they may arise from and contain self-duplicating submicroscopic particles. Darlington (1949*a*) further infers the existence of genetic sub-units in the centromere, which is in some respects analogous to the centriole, from its capacity for misdivision.

If Imai's lead were followed, a special term would be coined to designate the hypothetical genetic sub-unit in each visible, self-duplicating structure. To "plastogene" would now be added "kappagene," "centriogene" and "mitochondriogene"; and eventually there would probably be proposed "cinetogene" and even, alas, "gulletogene"! To recite this incomplete list of awkward and cacophonous terms is in itself almost enough to reject them. If they are to have a name at all, let them have a common name emphasising their common cytoplasmic localisation and their common gene-like properties. The most appropriate term, it seems to me,

is Winkler's (1924) old term "plasmatic gene," stripped of its more recently acquired special limitations and condensed to its more familiar form "plasmagene."

But we must not be led astray by our prejudice in favour of sub-microscopic units of heredity. The existence of plasmagenic sub-units in any one of the self-duplicating, cytoplasmic structures has not yet been established, while the possession of the genic properties of self-duplication and mutability for the gross structure is fully demonstrated in some cases. It therefore seems to me unwise to limit the term plasmagene to the possibly non-existent sub-units and deny it to the structure as a whole. If and when the genic properties are in any case shown to depend upon sub-units, then the term plasmagene should be shifted from the structure as a whole to its gene-like component parts. Meanwhile I shall employ the term plasmagene for those cytoplasmic structures known to manifest genic properties, however infelicitous this may seem when applied to bodies large enough to be microscopically visible.

(c) *Relation to the nuclear genes*

The proof that a difference in hereditary traits is due to a difference in cytoplasm necessarily involves the demonstration that the organisms manifesting the diverse traits have identical genomes. If they had diverse genomes, the observed difference in traits obviously might be due to the gene differences. From the identity in genomes the conclusion is often drawn that the cytoplasmic basis of heredity, the plasmagene, is independent of the genes. Yet this conclusion is neither logical nor defensible. The genes may constitute a necessary, but not a sufficient, factor in the formation, maintenance or reproduction of the plasmagenes.

How then can it be determined whether the plasmagenes are in any way dependent on the genes? Unfortunately, there is no invariably dependable method of obtaining an answer to this question. The only method that can be used is the method of gene substitution. With luck, this can prove that genes are involved, but it can never prove that genes are not involved. Let us make the point concrete with a few examples.

The plastids, which are the basis for at least some examples of cytoplasmically inherited variegation in plants, were long believed to be independent of the genes because diverse plastid types persist and multiply in association with the same genome. Renner's observations on *Oenothera* showed this view to be false (see Rhoades, 1946). He obtained hybrid plants carrying two kinds of plastids of diverse origin. In these hybrids both plastid types persisted and multiplied; but one kind did not develop or function normally. When the hybrids were selfed or crossed to certain other plants, the abnormal type of plastid recovered and functioned normally in some of the resulting genomes.

Thus, certain plastid types are adapted to definite intracellular conditions which the genes control. The illusion of gene-independence is dispelled when appropriate materials are investigated.

Interpretation of the relation of kappa to the genes in *Paramecium* passed through a similar history. The sensitives employed early in the work carried the *K* gene, as did of course the killers. Crosses between such materials showed no difference between killers and sensitives in any genes affecting the killer trait. Likewise, the irreversible destruction of kappa, transforming killers into hereditary sensitives, and the introduction of kappa into sensitives, transforming them into hereditary killers, indicated that kappa was independent of the genes. Only after four years did the lucky chance of finding strains carrying *k* reveal the hidden gene dependence: kappa disappears in the absence of gene *K*.

In view of this experience and of Renner's work on plastids, it should be clear that the failure to find a gene difference affecting a plasmagene is no guarantee that the plasmagene is gene independent (Sonneborn, 1943). Moreover, *a priori* considerations render improbable the possibility that such plasmagenes could persist and multiply except in cytoplasm of a definite constitution which could scarcely be free from decisive gene modification. I therefore doubt whether totally gene-independent cytoplasmic inheritance exists in any form, based on plasmagenes or otherwise.

However, the nature of the dependence of a plasmagene on the nuclear genes may not be as obvious as it seems. In the examples already discussed, the genes seem clearly to control the functioning or the maintenance, but not the initial production, of the plasmagene. The plasmagene disappears or manifests disfunction in cells of the "wrong" genotype, persists and functions normally in cells of the "right" genotype; but, once lost from the "right" genotype, it is not again produced. Cells with only defective plastids may have the same genes as cells with normal plastids, yet they do not produce normal plastids. *Drosophila* lacking sigma do not produce sigma, even when they have the same genes as sigma-bearing strains. Sensitive paramecia that lack kappa do not give rise to kappa, although they may have gene *K* and every other gene present in killer paramecia. The essential genes in these cases seem not to function in the initiation of the plasmagenes. On the other hand, the problem of whether the genes can initiate formation of a plasmagene is, in a sense, like the problem of discovering whether plasmagenes are ever gene-independent. Failure to demonstrate it may be due to methodological failure. As Waddington (1948) has pointed out, the very fact that a cell has developed in one way may make it practically impossible to detect whether it could have developed in another way. The internal conditions may no longer be comparable to the ones that prevailed when the alternative pathway was still a practicable possibility. While such considerations make judgments uncertain,

nevertheless, in the absence of evidence to the contrary, it seems unlikely that the genes can initiate production of plastids, kappa, sigma or the like.

The dependence of plasmagenes on nuclear genes raises two further questions about them : one concerning their relative importance and the other, their evolution. It has been maintained, on various grounds, that plasmagenes occupy in the cell hierarchy a rank lower than the genes (Beadle, 1949). Yet, so far as maintenance and normal functioning are concerned, the dependence of plasmagenes on genes is not obviously either greater than, or different from, the dependence of genes upon cytoplasm. Baltzer (1910) showed that certain chromosomes of the sea urchin, *Sphaerechinus*, could not be maintained in the cytoplasm of *Strongylocentrotus* ; and he and others subsequently reported a number of comparable cases in other organisms. Similarly, certain chromosomes are regularly eliminated from definite cells of *Ascaris* (Boveri, 1899) and *Sciara* (Metz, 1931) ; and one chromosome of a variety of *Vicia faba* is unable to survive in homozygous form in the cytoplasm of another variety (Sirks, 1931). The cytoplasmic conditions involved here may be, and probably are, more or less affected by genes. There is no evidence of a reverse effect of cytoplasm on the character of the chromosomes. In this sense, the cytoplasm may be subordinate to the genes. Nevertheless, it is still clear that chromosome maintenance depends upon specific cytoplasmic constitution just as the maintenance of plasmagenes depends upon the proper chromosome constitution. Genes and cytoplasm are mutually and specifically dependent upon one another for their maintenance.

The evolution of plasmagenes has been reflected upon by numerous authors for more than twenty years. A recurrent suggestion has been that plasmagenes and nuclear genes may undergo parallel evolution. Renner's report that genetically different plastids occur in different *Oenotheras* and that the different plastid types are adapted to different genomes is in agreement with the notion of parallel evolution. I have preliminary indications of the same sort of thing in *Paramecium*. Killers occur in two varieties of *P. aurelia*, varieties 2 and 4, which cannot interbreed at all. A killer race of variety 2 was freed of kappa and thereby converted into a sensitive. It was then exposed to the macerated bodies of a variety 4 killer. This method, it will be recalled, is successful in introducing kappa into *KK* sensitives of variety 4, thereby transforming them into hereditary killers. But the attempts thus far made to use this method for introducing kappa from variety 4 into variety 2 have failed. Although the matter requires much further investigation, it is possible that the kappas in these two varieties, like the chloroplasts in Renner's *Oenotheras*, depend on different genomes for their normal maintenance. The genome of the variety 2 race can maintain its own kappa, but it may be unable to maintain the kappa from variety 4. It would be easy to account for such a condition by assuming first a mutation of kappa and then a gene

mutation which could support only the mutant, not the original, kappa. Some such course of events might also underly other examples, previously mentioned, of incompatibilities between chromosomes and cytoplasm. In other words, selected and correlated mutations may occur in nucleus and cytoplasm in much the same way as must occur in hosts and parasites if their association is to be preserved.

(d) *Transmission and rate of reproduction*

Some plasmagenes are distributed to the daughter cells in an essentially random manner at cell division. Preer (1948a) has pointed out that randomly distributed genetic particles, multiplying at the same rate as the cells in which they lie, must eventually be lost from an increasing proportion of the cell progeny. How is it then that randomly distributed plasmagenes manage to persist? Kappa may be taken as an example of a plasmagene which is distributed practically at random during cell division. Preer (1948a) has shown in this case the existence of compensatory mechanisms. When the cellular concentration of kappa drops, its rate of reproduction rises; when the cellular concentration of kappa rises, its rate of reproduction falls. This serves to keep the concentration fairly uniform under most ordinary conditions and thus cancels the risks of loss by random distribution.

Some plasmagenes, on the other hand, are not randomly distributed at cell division. The cinetosomes or ciliary basal granules of *Paramecium* and other ciliates remain in fixed position. There they duplicate and, at fission, every animal gets its full allotment of cinetosomes. There is no danger of loss by this method of distribution.

Still other plasmagenes are as precisely distributed as are the chromosomes by mitosis. The centriole divides when the cell divides and each daughter cell gets one. The gullet of *Paramecium* buds off a new gullet which severs its connection with the parental gullet and migrates to the proper position in the daughter cell (Hertwig, 1889). In certain organisms, the chloroplasts are precisely and equally distributed at cell division. The mitochondria are in some cases equally distributed; they may even undergo precise reduction of number at the time of the meiotic mitoses (Wilson, 1916).

The various methods of distribution of plasmagenes are thus all well adapted to preserving them. Indeed, there seems to be no fundamental difference between plasmagenes and nuclei in respect to method of distribution at cell division. Most nuclei are of course distributed equally and precisely, like centrioles and certain other plasmagenes. When there are normally many nuclei in a cell, as in some ciliated protozoa, they are distributed in the same manner as kappa, *i.e.*, randomly.

As already implied, the reproduction of some plasmagenes is not necessarily synchronised with nuclear and cell division. Under some

conditions, chloroplasts may divide more slowly than the cells that contain them (Ternetz, 1912). This of course results in fewer chloroplasts per cell or in none at all. Similarly, kappa multiplies less rapidly than the paramecia under certain conditions of feeding (Preer, 1946) and of temperature (Sonneborn, 1946). Conversely, under other conditions of feeding and temperature, the rate of multiplication of kappa may be greater than that of the cell until the maximal concentration is attained.

These aberrancies in plasmagene reproduction are also paralleled by similar aberrancies in the reproduction of nuclei and chromosomes. As is well known, cells can divide without nuclear division, nuclei can divide without cell division, and chromosomes can duplicate without either nuclear or cell division. The phenomena do not seem to be different in principle as between plasmagenes and nuclear genes. Under normal conditions, in both cases, there is usually a neat correlation in replication rates to preserve the *status quo* ; under aberrant conditions, these correlations are disturbed and either the plasmagenes or the nuclear genes may be lost, often with fatal consequences in either case. There must be strong selection under normal conditions for correlated replication rates of all essential self-duplicating cell structures.

(e) Functions

Beadle (1949) and some workers on the plasmon (see review by Caspari, 1948) maintain that plasmagenes have relatively unimportant functions in comparison with the nuclear genes. According to a common form of this view (see Caspari), all essential activities of the cell are controlled by the genes, plasmagenes doing nothing but interfering with gene action. For example, Schlösser (1935), who subscribes to this view, showed that one of the plasmon effects in *Epilobium* is to alter the osmotic pressure of the cells and thereby to inhibit the action of certain genes. But the osmotic pressure is itself an hereditary trait, which in this case at least seems to be determined by the plasmon. Moreover, inhibitory or modifying actions are certainly not uniquely characteristic of plasmagenes, for genes also act as specific inhibitors or modifiers of the action of other genes. Finally, there can be little doubt of constructive, as opposed to interfering, action when respiratory enzymes are cytoplasmically inherited (Ephrussi *et al.*, 1949) or when the plasmagene is an essential and non-replaceable structure like a chloroplast, a centriole, a cinetosome or the like.

(f) Origin

A common criticism of plasmagenes is that they are not normal integral parts of the genetic equipment of the cell, but rather symbionts or parasites of extrinsic origin. Lindegren (in Sonneborn, 1946) and Altenburg (1946) have maintained this with respect to kappa ;

L'Héritier (1948) has considered the same possibility for sigma. There are certainly reasons for suspicion. Kappa is infectious when in concentrated suspension (Sonneborn, 1948*a*) and, unlike normal cytoplasmic constituents as at present conceived, it contains desoxyribonucleic acid (Preer, 1948*b*, and in press); moreover, most paramecia contain no particles comparable to kappa. Sigma too is infectious by the techniques of transplantation and injection (L'Héritier, 1948). Both Sonneborn (1948*a*) and L'Héritier (1948) have emphasised, however, that these particles are infectious only under conditions that could scarcely occur in nature. Because of this, Darlington (1949*b*) refers to them as "proviruses." In my opinion (Sonneborn, 1948*a*, 1949), the question is largely verbalistic and tends to become academic. I believe Darlington, L'Héritier, and I are agreed that kappa and sigma are borderline cases and that, regardless of their origin, they are of genetic significance because they are normally transmitted only by heredity.

One cannot but be impressed by the fact that practically every self-duplicating structure occurring within cells has at one time or another been considered a symbiont or parasite. For example, chloroplasts and mitochondria have been interpreted as symbionts (see review by Buchner, 1921). Even the nuclear genes have not been spared. Thus Wallin (1927) claims to have demonstrated that mitochondria are symbiotic bacteria and he believes that they provide the chromosomes with the new genes necessary for evolution. However interesting they may be, all such speculations as to the possible external origin of self-duplicating intracellular bodies are, from the point of view of the present genetics of the organism, outweighed by the fact that at least some of them, such as plastids and other cell structures, are now normal parts of the genetic equipment of the cell.

(g) Occurrence

It has often been urged that very few plasmagenes with detected effects on hereditary traits are known, particularly in animals. By comparison with the number of known nuclear genes, this is certainly true. Yet it must be remembered that the detection of plasmagenic effects may be beset with special difficulties. Unlike lethal nuclear gene mutations, lethal plasmagene mutations could not be recognised at all. The phenomena of diploidy and meiosis, which preserve and reveal nuclear gene mutations, even lethals, have no known counterparts that would render possible the detection of lethal plasmagene mutations. If plasmagenes control very fundamental processes that cannot be altered without disastrous consequences, they would seldom be detected. We may not often expect so fortunate an arrangement as the dual system of respiration in yeast, without which it would not have been possible to discover the cytoplasmic inheritance of respiratory enzymes (Ephrussi *et al.*, 1949). Nevertheless, it is obvious

—even without employing ordinary genetic methods—that visible plasmagenes form much of the essential equipment of the cell. These are indeed widespread, common, even ubiquitous.

III

The specificity of the physical basis of cytoplasmic inheritance is in some cases controlled, not by the plasmagenes or the cytoplasm, but by the nuclear genes. This is commonly believed to be the case for the diverse cell types that arise during the development of higher animals from the fertilised egg, and that persist during repeated subcultures *in vitro* or in grafts. However, there is little critical evidence upon which to base a well-founded genetic interpretation of persistent developmental differentiations and, as a result, a number of fundamentally different interpretations have been suggested or repeated in recent years (Darlington, 1944 ; Sonneborn, 1943, 1947*b*, 1948*b*, 1949 ; Spiegelman, 1946 ; Waddington, 1948 ; Wright, 1941, 1945). The obvious limitations of genetic analysis of somatic cell differences stress the need for a comparable situation in material better suited to genetic analysis. This seems to be provided by our study—still in progress—of the determination and inheritance of antigenic types, or serotypes (Sonneborn, 1947*b*, 1948*b* ; Sonneborn and Le Suer, 1948 ; Beale, 1948 ; Sonneborn and Beale, 1949 ; and Sonneborn *et al.*, unpub.).

(a) *The main facts on determination and inheritance of serotypes in P. aurelia, variety 4*

After paramecia of a certain strain are injected into a rabbit, the rabbit's serum, even in high dilution, immobilises or paralyse paramecia of the same strain. This is a specific antibody-antigen reaction, for dilute antisera obtained from different rabbits injected with different strains of paramecia may be capable of immobilising only the homologous strain, *i.e.*, the one injected into the rabbit from which the serum was obtained. Each serologically distinct strain of paramecia is known as an antigenic type or serotype.

Each stock (consisting of the vegetative and sexual progeny derived exclusively from one homozygous individual) gives rise eventually to a series of diverse serotypes. The serotypes thus far found in stock 51 have been designated A, B, C, D, E, G, H and J ; those in stock 29 are A, B, C, D, F, H and J. F has not yet arisen in stock 51 and neither E nor G has arisen in stock 29. Moreover, serotypes A, C, D and H are not identical in the two stocks : antiserum against any one of these types, from either stock, will immobilise animals of the corresponding type from the other stock, but minor differences between the corresponding types are clearly demonstrable by serologic methods. Further study may reveal that the other types which occur in both stocks are also distinguishable, and it is practically

certain that more types will be found. Differences in the spectrum of possible serotypes, comparable to those that distinguish stock 29 from stock 51, are also found among the other stocks of variety 4 obtained from different sources in nature. The fact that every animal in each stock belongs to some serotype, indicates that these traits are normal and indispensable. As similar serotypes occur in stocks collected from Asia and America they are apparently ancient characters of the species.

The immobilisation of each serotype by its homologous antiserum seems to involve a single distinctive major antigen or antigen complex, which may be designated as its "specific immobilisation substance" (*SIS*). For example, serotype A is apparently not immobilised by the antibodies that immobilise any other serotype. Hence, the *SIS* of serotype A is not present, or at least it seems not to function in immobilisation, in any other serotype. Although the stock as a whole is capable of producing a whole spectrum of *SIS*, the presence of any one of them in any individual excludes more or less completely the presence of any other one. In a few serotypes, however, there is present not only the *SIS* of this serotype but also the substance which acts as a *SIS* in another serotype. The latter seems to be present in large amount, but it does not function in immobilisation. As will appear, mutual exclusion of specific immobilisation substances is of decisive importance in the theoretical interpretations.

Exposure of any serotype to its immobilising antiserum transforms it into other serotypes. Antiserum, along with temperature and other conditions, can direct the transformations to a particular type. For example, serotype D of stock 29 can be transformed by anti-D serum to type B at 32°, to type H at 20°, in each case about 95 per cent. of the treated individuals transforming. Some, indeed probably all, of these transformations are reversible. In stock 51, A and B are repeatedly convertible one into the other, and all other serotypes can easily be transformed to A or to B, as desired. In stock 29 serotypes B, F and H are readily interconvertible and any other serotype can be transformed to any of these. Most of the remaining transformations are not yet under experimental control, but they may yet be achieved. It seems therefore that irreversible losses of potencies do not take place in the process of transformation.

The serotypes are inherited under standard conditions of culture (26° to 27° C. with enough food to permit one fission per day). Spontaneous changes of serotype rarely occur under these conditions. When spontaneous or induced changes do occur, the new types are inherited thereafter under the standard conditions of culture. Crosses made among different serotypes of the same stock reveal no effective gene differences among them: all the serotypes of one stock are identical in genes. As this suggests and as further analysis shows, the serotype differences follow the cytoplasm in inheritance. Hereditary transformations of serotype are thus cytoplasmic "mutations" of hitherto unknown type.

But the genes also play a part in the control of serotypes as appears when different *stocks* are crossed. It will be recalled that the specific immobilisation substance of serotype A is not quite identical in stocks 29 and 51. Crosses between the two stocks show that the specific difference between their A substances is controlled by a pair of allelic nuclear genes with the 51A gene dominant over the 29A gene. This is the basis for concluding that nuclear genes control the specificity of the physical basis of cytoplasmic inheritance in these antigenic traits.

Single gene differences have also been found to control other differences between the stocks. For example, serotype F is one to which any serotype of stock 29 can be readily transformed ; but we have not succeeded in obtaining type F under any conditions in stock 51. The capacity to transform to type F under the conditions employed depends upon a gene present in stock 29 and absent from stock 51. Serotype H is another to which any serotype of stock 29 can be easily transformed under defined conditions ; but methods for achieving this in stock 51 are unknown. The capacity to transform to type H under the conditions employed depends upon another gene present in stock 29 and absent from stock 51.

The results with serotype H are instructive. They were obviously parallel to the results with type F and we at first believed they meant that genes for the F and H specific substances were lacking in stock 51. It was not until much later that type H was found for the first time in stock 51. Obviously the gene we were following has nothing to do with the capacity to form the H substance ; it merely controls the response to particular transforming conditions. For example, at low temperatures, stock 29 serotypes transform to types F and H while stock 51 serotypes transform mainly to type B. In view of these new findings the F gene previously followed is probably of the same sort and tells nothing about the capacity of stock 51 to yield type F.

Indeed other evidences from serologic and genetic experiments indicate that stock 29 also has the genes required for control of the specific E and G substances, although these serotypes have never been found in stock 29. I therefore believe that stocks 51 and 29 both have the genotype required for production of all the serotypes A to J. On this view the differences between the stocks are of two kinds : first, corresponding serotypes are slightly different as a result of different specificity alleles ; second, corresponding serotypes are called forth or stabilised under different conditions, the response system being controlled also by genes.

To summarise, the main facts brought out by this survey are : (1) Each homozygous stock is characterised by a spectrum of possible serotypes. (2) Different stocks are characterised by different spectra of possible serotypes. (3) The specific immobilisation substance of each serotype more or less completely excludes the presence or functioning (in the immobilisation reaction) of any other specific immobilisation substance. (4) Environmental conditions (including

homologous antiserum) bring about directed transformations of serotype. (5) Many, possibly all, of the transformations are reversible, indicating that no potencies are lost in the process of transformation. (6) The differences among the serotypes in any one stock are due not to genic, but to cytoplasmic, differences which are inherited under the standard cultural conditions. (7) The specific differences between corresponding immobilisation substances in different stocks are determined by allelic genes. (8) Each stock of variety 4 probably has a full set of specificity genes for all the serotypes found in the stocks of this variety. (9) The stocks differ in other genes controlling the response to particular transforming conditions (*i.e.*, which serotype will arise).

(b) *Comparison of the serotype and killer systems of inheritance*

The system of determination and inheritance of the serotypes probably reflects a fundamentally different physical basis and mechanism of cytoplasmic inheritance, because it differs so strikingly from the system exemplified by the killer trait and set forth in part II of this paper. To be sure, the two systems are alike in some features : in both, traits are cytoplasmically determined and inherited ; in both, environmental conditions can bring about hereditary changes ; in both, the cytoplasmically inherited traits depend for their maintenance on nuclear genes. But there the resemblances end.

The differences appear to be of four main kinds. First, the specific type of killer trait is controlled by the kind of kappa present, not by the genes, as Dippell's analysis of kappa mutations demonstrated. On the other hand, the specific type of A immobilisation substance is determined by the nuclear genes. Second, transformations from killer to sensitive are readily made irreversible, while transformations of serotype are not. Third, the various types of killer character are not mutually exclusive, as Dippell's study of the mutants shows : different kinds of kappa can coexist in the same animal and its progeny, each kind of kappa controlling production of its corresponding kind of paramycin. In the serotypes, on the other hand, two kinds of specific substances, both functioning as immobilisation substances, cannot coexist and maintain themselves in a line of descent. They are mutually exclusive. Fourth, the physical basis of the killer trait lies in the visible, Feulgen-positive kappa particles. Search for such particles associated with the serotypes has failed to reveal them. These fundamental differences between the killer and serotype systems must be taken into account in attempts at interpretation.

(c) *Interpretations of the serotype system*

(1) *The hypothesis of gene-initiated plasmagenes.*—Cytoplasmic inheritance is naturally explained by plasmagenes, just as nuclear inheritance is by chromosomal genes. The fact of genic control of specificity in

the serotype system restricts plasmagenic interpretations to forms different from the one discussed in the first part of this paper. In this section, we consider the hypothesis that the specificity-controlling genes produce the specific immobilisation substances which are the plasmagenes. This was adopted by Sonneborn (1948*b*) and Beale (1948) as a working hypothesis and it had the merit of leading to the discovery of a number of the facts. It is based on an idea which has been considered by Wright (1941), Darlington (1939, 1944), and Spiegelman (1946), and which is at least as old as De Vries' pangenesis.

On this hypothesis, the genic origin of the specific substances is suggested by the genic control of specificity. The reversibility of transformations is due to the permanence of the genic source of the plasmagenes. The efficacy of specific antiserum in bringing about transformations is due to interference with plasmagene reproduction as a result of the combination of plasmagene with specific antibody. To account for mutual exclusion and the environmental control of the direction of transformation, an additional assumption is required. As this requirement is common to several of the hypotheses, it will be discussed later.

The hypothesis of gene-initiated plasmagenes meets a serious difficulty in accounting for the results of the cross between 51A and 29A. It will be recalled that the 51A gene behaved as a dominant. This means that all of the F_1 were of serotype 51A, even those (derived from one member of each pair of mates) whose cytoplasm was carrying the full measure of 29A substance before fertilisation. This seems to be inconsistent with the plasmagene hypothesis. If the specific 29A substance is a plasmagene, it is by definition capable of multiplication and should not disappear. To be sure, in the hybrid the 51A substance should also arise under the action of the 51A gene. A mixture of 51A and 29A substances is therefore expected in the vegetative progeny. But the 29A substance is found only in one member of each conjugant pair and only for a few fissions, after which it can no longer be detected. Even if dominance in this case is understood as suppression of the action of the 29A gene by its 51A allele, the results are not explained. This would merely cut off the further production of 29A plasmagenes by the gene; it would not account for the apparent failure of multiplication by the plasmagenes already present.

In view of the F_1 results, the observations in the F_2 are not surprising. Each clone develops the serotype corresponding to its genes. Those that carry the 51A gene remain 51A; those that become homozygous for the 29A gene lose the 51A substance in a few fissions and transform to 29A. This shows that the results in the F_1 could not be due to suppression of the 29A plasmagene by the 51A plasmagene, for the reverse occurs in the F_2 . Moreover, there is no obvious difference in the rate at which the two opposite changes occur.

Thus the 29A substance quickly disappears in the presence of the 51A gene, even when the 29A gene is also present ; and the 51A substance disappears just as quickly in the presence of the 29A gene, when the 51A gene is absent. This can hardly be interpreted otherwise than as a conspicuous failure of the gene-initiated plasmagene hypothesis. And a more favourable opportunity for such plasmagenes to demonstrate their existence could scarcely be conceived.

Negative evidence is of course never quite convincing. Yet this is the second failure of the hypothesis. A few years ago, Spiegelman, Lindegren and Lindegren (1945) claimed that the adaptive enzyme, melibiozymase, in yeast was maintained and multiplied in the presence of substrate and in the absence of the gene required for its initial formation. If this claim had been confirmed, it would have gone far toward establishing the existence of gene-initiated plasmagenes. Naturally the work attracted an enormous amount of interest and was the basis for widespread acceptance of the concept. However, attempts to repeat the observations have failed (Lindegren and Lindegren, 1946).

Our own failure is even more striking because the assumed plasmagene was lost in the F_2 , in the presence of a gene with an effect almost identical with that of the controlling gene ; and because it was lost in the F_1 *even in the presence of the controlling gene itself*. Thus, if gene-initiated plasmagenes are involved here, they must also depend on the same gene, subject to the rules of dominance, for their maintenance. Why this should be is not at all evident. It would seem more likely that *other* genes would control the cell conditions affecting survival or multiplication of the plasmagene. If the same gene that is assumed to be producing the plasmagene must *continually* act to assure its survival and multiplication, it might as well be assumed that the specific substance is formed under gene action without the intervention of plasmagenes at all. With respect to the serotypes, therefore, the existence of gene-initiated plasmagenes is no longer even a useful assumption.

The other evidence cited for gene-initiated plasmagenes is the apparent autocatalytic increase of the substances involved. But autocatalytic increase is well known in systems devoid of a genetic component, so evidence of this sort cannot alone be considered decisive. We are thus driven to conclude that critical evidence for gene-initiated plasmagenes is entirely lacking.

(2) *The hypothesis of precursor as plasmagene.*—Wright (1945) suggested that genes may act by conferring specificities on plasmagenes. The specificity genes in serotype determination could act in that way, converting an indifferent precursor, with plasmagenic properties, into a specific immobilisation substance, but one that *lacks* plasmagenic properties. With this hypothesis the facts that rule out gene-initiated plasmagenes are consistent. The detected trait, *i.e.*, the specific immobilisation substances, would depend directly on the genes present,

just as they do. Unfortunately, however, the hypothesis effectively puts the plasmagene beyond the reach of present evidence, because the precursor is not directly detectible by any methods yet used.

Nevertheless, something can be said about the precursor, particularly as to whether there is one common to all the immobilisation substances or whether there is a unique precursor for each specific substance and its analogues in different stocks. Since the purpose of postulating a plasmagene is to account for cytoplasmic inheritance, there must be a different plasmagene for each of the alternative cytoplasmically inherited traits. Hence, in each stock one must assume a different precursor for each of its possible specific immobilisation substances.

This leads to predictions that might eventually be tested. First, it should be possible to remove one serotype from the spectrum of possibilities of a stock by destroying one of the precursors, as kappa was destroyed. Second, if the serologic properties of the specific immobilisation substances are determined in part by the nature of the precursor, then mutations of the plasmagene should be detectible. Third, in the same way corresponding precursors in different stocks must sometimes differ and the sort of observations made in the cross of 51A by 29A should show whether precursor multiplies in the absence of its original specificity gene. The two latter predictions could be tested only by finer serologic distinctions than any yet recognised in our work.

(3) *The hypothesis of variable gene activity.*—Kimball (1947) suggested that the rate at which a gene functions might vary directly with the cellular concentration of its direct or indirect product. This would result in cytoplasmic inheritance, for high concentration of such a product would call forth from the genes the action required to maintain it in high concentration. On this view the specific immobilisation substances themselves, or specific precursors of each, are the stimuli for accelerating the activity of the controlling genes. The transforming action of antiserum would then be interpreted as due to the failure of specific-substance-antibody complex to stimulate the gene in the way done by the specific substance alone.

(4) *The need for an additional assumption.*—The three hypotheses we have considered all fail, without an additional assumption, to account for two of the most striking facts in the serotype system. One of these is the mutual exclusion phenomenon, *i.e.*, the fact that in each of the serotypes only one of the several potentially producible substances is present as a specific immobilisation substance. The other is the environmental control of the direction of transformation, *i.e.*, the fact that a particular specific immobilisation substance arises under given transforming conditions. To account for these two facts, either one of two additional assumptions may be made.

First, competitive processes may be assumed (Sonneborn, 1948b). Depending on the hypothesis, the competition would be among the

plasmagenes or among the gene products that stimulate gene activity. The competition could be for sources of energy, for simpler substances used in synthesis of the specific substances, or for position in the cell. Control of the direction of transformation by environmental conditions would then be an expression of environmental influence on the competitive process.

Second, the presence of any one of the specific substances (or of a precursor of it) in high concentration may inhibit some step in the synthesis of all the other specific substances (Delbrück, in Sonneborn and Beale, 1949).

Not only does such a system of mutual inhibition account for mutual exclusion and directed environmental transformations, but, as Delbrück clearly showed, it also accounts for cytoplasmic inheritance without resort to plasmagenes. For, so long as the inhibitor is present in high concentration, it is perpetuated by excluding the alternative substances.

Doubtless many variations on this theme could be imagined. For example, Waddington (1948) has indicated how competition for substrates, combined with autocatalysis of the products, could yield a system capable of developing along any one of several mutually exclusive lines and, at the same time, automatically perpetuating the alternative actually realised.

(5) *Conclusions as to interpretations.*—Of the several hypotheses considered as explanations of the serotype system, the hypothesis of gene-initiated plasmagenes has been strongly discredited; but the others are all more or less satisfactory. Two considerations favour the hypothesis of gene-specified plasmagenes (supplemented with a competition or inhibition assumption). First, some of the discoveries made in the serotype work were predicted on the basis of plasmagenes and competition; these assumptions have therefore justified themselves as a working hypothesis. Second, plasmagenes are known to be the basis of cytoplasmic inheritance in a number of cases, as pointed out in the first part of this paper, while not a single example of cytoplasmic inheritance has yet been shown to be based on variable gene activity or mutual inhibitions or competition and autocatalysis. On the other hand, either of the two latter alternatives may be preferred because they are simpler, dispensing both with plasmagenes and variable gene activity and retaining only an accessory assumption required by the other, more complex hypotheses. However, there is no critical evidence that would exclude any one of these hypotheses. If any one of the three predictions set forth in the discussion of the precursor-plasmagene hypothesis were verified, this would constitute such critical evidence.

This will, I am afraid, seem like a most unsettled and unsatisfactory state in which to leave the remarkable system of serotype determination and inheritance, particularly after such a lengthy theoretical discussion. But we are studying a type of cytoplasmic inheritance never before

subjected to this sort of intensive experimental analysis. And the investigation is still young ; theoretically decisive results may yet be expected.

The main justification of the discussion, however, is its possible relation to normal developmental differentiation. The spectrum of serotypic possibilities inherent in each stock of paramecia may bear more than a superficial resemblance to the spectrum of cellular possibilities inherent in the fertilised egg. To the nine facts of the serotype system listed on pp. 25 and 26, there are either known or probable parallels in normal developmental differentiation, if the inductors and evocators of development are substituted for the environmental agents used to transform *Paramecium*. Only concerning the fact of reversibility can there be serious doubt and recent work of Rose (1948) and others shows that even this doubt should not be too strong.

IV. SUMMARY

Cytoplasmic inheritance is one of the capital facts of biology. It occurs in at least two forms that seem to be fundamentally different. One of them is based on self-duplicating, mutable, cytoplasmic particles—the plasmagenes—which depend on the nuclear genes for their maintenance or normal functioning, but not for their origin or for their specificity. These plasmagenes, in different cases, vary in size from microscopically visible particles down to submicroscopic particles probably of the same order of size as the genes. There are indications that at least some of the larger plasmagenes contain or consist of smaller genetic units. Plasmagenes are probably never independent of the genes, but this does not support the conclusion that they occupy in the cell hierarchy a position inferior to the genes. Genes and cytoplasm are mutually interdependent for their maintenance and normal functioning. Evolution may proceed by parallel but independent mutations of nuclear genes and plasmagenes. Plasmagenes have varied methods of transmission and varied rates of duplication, but in all cases the system is well adapted to their preservation. Far from merely interfering with normal gene action, plasmagenes control vital and constructive activities. While the origin of plasmagenes is obscure and may in some cases be external, some are at present normal and important parts of the cell organisation. Many examples of this sort of plasmagene are known and they may occur even more widely than is yet evident.

About the other form of cytoplasmic inheritance, manifested by serotypes of *Paramecium* and the somatic cell differences in higher animals, much less is known. This form is distinguished from the first one in several ways, chief among which is the genic control of the specific nature of the character that is cytoplasmically transmitted. Although a well-defined system of facts is known for this type of cytoplasmic inheritance, its physical basis and mechanism remain to

be discovered. Of the various possibilities, that of plasmagenes produced by genes seems to be excluded, but another type of plasmagene hypothesis and other hypotheses in which plasmagenes play no part remain to be tested.

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FIG. 1.

FIG. 1.—A sensitive *Paramecium*, Giemsa stain, showing macronucleus and a food vacuole containing bacteria, but no kappa particles in the cytoplasm. $\times 1430$.

FIG. 2.

FIG. 2.—A killer *Paramecium*, Giemsa stain, showing the macronucleus and the large number of kappa particles in the cytoplasm. $\times 1430$.