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Antigens and Antibodies as Cell Phenotypes

How does cell heredity change when host-graft relations are altered or antibody formation is stimulated?

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The brilliant experimental successes of recent years, which have shown a basis for the behavior of tissues on transplantation in immunological and genetic phenomena, seem now to be ranged in two sharply contrasted bodies of data. On the one side, the rigorous "laws of transplantation" demand a community of genetic constitution between host and implant for the graft to take (*1*); the antigenic constitution of the tissue in this regard is as directly related to the specific genotype as are the classic immunological blood groups. Contrasted with this definite picture are the experiments in which tolerance is induced, or histocompatibility barriers are overriden, or tumors are enhanced in their growth or adapted to their hosts. Similarly, when the cellular basis of antibody synthesis is considered, analogous problems in cell heredity appear. It is the object of this article (*2*) to consider how far the genetic analysis in quite another field—that of the serotypes of ciliate Protozoa—may be helpful in unifying these contrasts and also to explore what known mechanisms operating in the chromosomes may be common to both systems.

Change in Transplantation Specificity

A genetic analysis of differences in histocompatibility factors (*3*), as Snell has called them, is made by determining the frequencies with which tissues from inbred strains of mice will become established as grafts in the progenies of hybrids between them. In the F_1 hybrid, both parental types of graft will take. In the backcross, or in the F_2 generation, the frequency of take is a measure of the different genetic types segregating out according to Mendelian expectations. Thus, in the mouse, largely as the result of the work of Snell and his colleagues at Bar Harbor, at least 15 loci are believed to be concerned with these differences. Three have been more closely analyzed than the others, and in the case of the so-called H-2 locus, have now been dissected into a system of pseudoalleles, also responsible for blood antigens. The conventional precision of the genetic picture is remarkable.

This situation, straightforward in principle even though complex in its details, becomes involved when experiments of another kind are considered. The initial discovery of the phenomenon we owe to Barrett and Deringer (*4*). They found that tumors, after a passage through F_1 hybrids between their strain of origin and some other, will undergo a change in the frequency of take when tested in the F_2 progenies or in backcrosses of the two strains. Moreover, a specific relation to the nature of the F_1 hybrid in which the change occurred was suggested: the increase in compatible grafts occurred only with F_2 or backcross progenies involving the same two strains.

Hauschka (*5*) extended this description in two ways: he found cases of change, after F_1 passage, in which the specificity of the graft was increased rather than decreased, and—more important—he interpreted the numerical data as showing a change in the number of histocompatibility gene differences between tumor and foreign strain. With the discovery of a correlation between heteroploidy of tumor chromosomes and an increase in host range on transplantation, it became apparent that selection of favored antigenic types among the population of cells in the implant could occur, and that it would be dependent on the constitution of the host (*6*). This interpretation of "immunoselection" was extended easily to the Barrett-Deringer phenomenon, in place of their original term, "adaptation." But this extension seems now to have been incorrect.

The recent important studies of Eva and George Klein (*7*) go far towards excluding the selection interpretation. The Kleins established a number of sublines, derived from small inocula of a single tumor, and made comparisons of frequency of take in F_2 populations between these and lines derived from the same tumor after passage through F_1 hybrids. In this material a direct test could be made of the hypothesis that cells of the type predominant after F_1 passage were already present in the original population and were merely selected out by the F_1 hybrid passage. Mixtures of the two types of cells (one "adapted," 70 "original") tested in the segregating F_2 population showed frequencies characteristic of the "adapted" type; hence, if these cells had been present even in relatively low numbers in the original tumor, no effect of the F_1 hybrid passage could have been detected.

Moreover, by short-term experiments in an Algire diffusion chamber (*8*), the Kleins were able to show that the effect occurred before displacement of one type by the other on a selective basis could operate. Since it is supposed that no cells pass through the walls of the diffusion chamber, these experiments also

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indicate that the change does not require cell-to-cell contact but may be mediated by humoral factors, in contrast to the homograft reaction itself (*8*). The Kleins, following a suggestion by Medawar regarding tolerance (*9*), incline towards the view that the F_1 hybrid effect may be a change in the reaction of the tumor to the antibodies it provokes in the host; they mention experiments with preimmunized animals as evidence that the change involves, not a difference in the antigenic composition, but rather an increased resistance to isoantibodies.

Serotypes in Paramecium as a Possible Model System

These two groups of experiments—the one showing orthodox genetic regularity, the other indicating a genetic lability—provide a model of a now recurrent problem in transplantation experiments. They present a dilemma—that of distinguishing between genotype and phenotype—which is not new to genetics (*10*). As a guide for such an effort, the analysis of possibly parallel cases in the ciliate Protozoa, especially *Paramecium,* by Sonneborn, Beale, Preer, and their associates (*11*) may be examined. These systems present obvious analogies, which have been recognized and mentioned on several previous occasions (*12, 13*).

In *Paramecium,* the criterion of antigenic constitution originally used by Sonneborn is the immobilization of the cell when exposed to its specific antiserum. Each clone therefore has its own serotype. Latterly, using fluorescein-labeled antibodies, Beale and Kacser (*14*) have shown that the antigen-antibody reaction takes place at the surface of the cell.

The study of different strains disclosed a diversity of antigenic constitution, and with the rich experimental techniques available in *Paramecium,* it was shown that the inheritance of the serotypes could be cytoplasmic in certain crosses but in others followed a strict Mendelian pattern. The apparent contradiction was beautifully resolved by proof that the same genotype could be compatible with the expression of a variety of different, in most cases mutually exclusive, cytoplasmic states. Take, for example, a clone of *Paramecium* exposed to its own antiserum; the immobilized individuals recover and are allowed to grow. Now, when the paramecia are tested, although the reproduction has been purely vegetative and the genotype is constant, the antigenic constitution has changed and is stabilized in a new form. These serotype transformations occur as a response to a wide variety of environmental stresses; the weight of opinion now tends towards the conclusion that any factor upsetting the general metabolism of the cell may begin the process and that its course in a given serotype during the ensuing cell divisions (during the process of differentiation, so to speak) depends on the environmental conditions (nutrition, temperature, and so on) for cell metabolism. When all these conditions are specified and remain constant, proper analysis of crosses shows a number of loci each represented by a range of alleles, each allele being responsible for a special serotype. Moreover, studies of heterozygotes afford evidence for the independent action of each allele present.

In a *Paramecium* of a specified genetic constitution, therefore, the serotype manifested represents the activity of one of a group of loci capable of affecting its antigenic constitution. Which locus is expressed in the cytoplasmic state depends on the constellation of factors at the time the pattern of antigen synthesis is set. The possible nature of the response in the nucleus is discussed in a subsequent section; yet it should be noted here that the analysis of phase change in the *Salmonella* antigens (*15*) has shown, by transductive techniques, that these antigenic changes are governed by a nuclear event in a system which also involves the mutual exclusion of antigens.

Change in Tumor Host Specificity

This analysis provides a model of cell heredity maintained through the cytoplasm, yet withal based on orthodox Mendelian principles. The possibility of an analogy between these cases and those encountered in the immunogenetic systems of mammals has not been entirely disregarded in previous discussions. However, if one takes the Barrett-Deringer phenomenon as a point of departure, a more explicit analogy may be drawn, which has consequences for future experimental design.

The homograft reaction, like the serological test, gives a first-order analysis of the specific antigens present in a cell type. In contrast to the situation described in the *Paramecium* serotypes, however, each of the antigens responsible for the homograft reaction appears to manifest itself independently of the others; the similarity to such immunogenetic cases as the blood groups is obvious. The different loci concerned are not all equivalent in their influence: the H-2 locus (the most studied), at which some half-dozen separate antigens have been shown from the different alleles (*3*), is the determinant in any situation where differences exist for it between graft and host. The other, weaker loci require special conditions of cell dosage, preimmunization of the host, and so on for unequivocal demonstration, as Snell (*16*) has found.

In the present context, it is important that these are the factors presumably affected by the F_1 passage; for the Kleins (*17*) have found no evidence of an F_1 effect in strains of tumors differing at the H-2 locus. It therefore follows that the weaker systems are those with which we are presently concerned, and the indication that preimmunization may obliterate the distinction between an F_1-treated tumor and its original type signifies that the effect of the passage is somehow on the quantitative relations of the immunological response. Thus it is conceivable that the effect of the passage on a specific locus is to change its activity.

In the *Paramecium* serotypes, transformation consists in the replacement of the products of the activity of one locus by those of another. It therefore consists of two phases: the triggering of activity at a locus and the displacement of the accumulated product of the old locus. The mosaic character of the histocompatibility antigens limits the analogy with the *Paramecium* system: the possible occurrence of competing steady states, so important in the discussions of the cytoplasmic states in *Paramecium,* does not concern us here. The question is the mechanism whereby change in the effective amount of a single antigen may occur. In terms of the *Paramecium* analogy, this is the initial triggering reaction, which occurs in the parental-strain tumor cell that is implanted in the F_1 hybrid.

Since the Barrett-Deringer effect shows a high degree of strain specificity, the influential factors in inducing the change are most reasonably sought in some immunological reaction, involving either the antigens themselves or some substances complementary to them. These must be supposed to enter the cell and, by cross-reacting with the antigen-producing system at the critical locus, to set up a new condition of cell heredity with respect to the histocompatibility effects at that locus. Formally, this is comparable to a directed somatic mutation, and the value of the *Paramecium* anal-

ogy lies precisely in the fact that it dissolves the formal terminology and necessitates more concrete thinking.

One line of speculation is the following: The loci manifesting the Barrett-Deringer effect are, as has been said, all "weak" loci—a condition interpretable as resulting from a tendency not to release antigen (or to form just enough to maintain what is needed for the cell economy). Hence, the formation of antibodies, whether circulating or cell-bound, lags in these types, and the histocompatibility barrier is easily vaulted. The exposure of cells containing such loci to the foreign antigens of the F_1 hybrid allows competitive conditions to be set up, in which the cytoplasmic state for the particular locus is changed. Either by the formation of antigens of a different type or by a change in the rate of antigen formation, the ability to elicit the homograft reaction is reduced, and this leads to an apparent decrease in the number of histocompatibility differences manifested in the test cross; or the ability to elicit the homograft reaction may be increased at a locus where the response was subliminal in the original line, before the F_1 passage, and this leads to an apparent increase in the number of factors required.

An alternative line of speculation emphasizes a possible increase in the resistance of the cells to the antibodies they elicit. This point of view, already envisaged by Klein in terms of "tolerance," suggests as a mechanism the kind of zone effect considered as a possibility for explaining the nonspecificity of heteroploid tumors (*18*) and elaborated for certain cases by Feldman and Sachs. Here the quantitative relations between antigen and homograft antibody are believed to be so changed that the amount of antigen in the cell as compared with the antibody available in its environment exceeds or fails to reach a critical ratio for occurrence of the homograft reaction.

Whichever of these possibilities is shown to be the actual mechanism (experimental tests suggest themselves, in which Snell's isogenic resistant lines are utilized), the essential mechanism of the adaptation in either case could be the activation of a new cytoplasmic state, in terms of the *Paramecium* analogy, but differing from *Paramecium* in that each locus conditions a state relatively autonomous of the factors at other loci. In both cases, the mechanism consists in the response of the cell to an environmental stress by the establishment of a new pattern of synthesis, stable under the new conditions.

Actively Acquired Tolerance and Enhancement

Now it is appropriate to consider whether the analogy with the ciliate serotypes can hold for other types of change in transplantation specificity. Those that come immediately to mind are the tolerances induced either in tumors, by implantation into organisms whose immunological system is not yet functional, or in immature organisms, by the implantation of foreign tissues. These are recognized as changes in the heredity of the cells in which they occur. Together with them we may consider the phenomenon investigated by Kaliss (*19*)—the enhancement of tumor growth after implantation in foreign-strain animals previously inoculated with frozen-dried preparations from the tumor. This also may be an effect not on the immunological system of the host but on the nature of the tumor cell itself. An interesting relationship exists between the time of inoculation and the time of implantation; only after the immune response tails off does the enhancement appear. This phenomenon, like certain other adaptations, seems not to be permanent but to be reversible after a number of transfers and to involve the H-2 locus.

Many of these phenomena may be susceptible of explanation in terms of "immunoselection," as Hauschka has used the concept—to account for the displacement of specific diploid by nonspecific heteroploid tumor stem lines. Yet the existence of one case in which the selection hypothesis has been disproved makes it advisable to consider in these others, also, whether the concept of the genetically controlled cytoplasmic state may not be useful.

The enhancement phenomenon, with its relation to the immunological system of the host, offers in some ways a more direct parallel to the *Paramecium* cases than does the F_1 hybrid change. For here there are present antibodies to the tumor which, by their reaction with the tumor antigens under conditions in which the tumor cell is not destroyed, trigger the establishment of a new compatible cytoplasmic state for the loci of histocompatibility difference. It should be noted that Kaliss has not yet found any permanence of what he believes to be the enhancement change; this difference from the Barrett-Deringer phenomenon may be a characteristic of the H-2 factors involved, or it may indicate a real difference in mechanism (such as antibodies involved for enhancement versus antigens in the F_1 hybrid).

As already mentioned, Klein has considered the possible relationship of the Barrett-Deringer phenomenon to the induction of tolerance towards foreign-strain tissues in animals implanted with the foreign lymphoid or bone-marrow cells while in the fetal state, or when new-born (*20*). The original case in cattle (*21*) contains the essentials: Twins different in their genetic constitution, but having had a common placental circulation, turn out to be mosaics (or chimeras) with respect to their blood cells. Each of the twins has cells of two blood types, representative of their respective genetic constitutions; thus, each genotype is now tolerated in the foreign host. When skin transplants between such twins are made, instead of being rejected, they are maintained. In a series of admirable investigations (*20*), the phenomenon has been explored in detail in rodents and in fowl, with the conclusion that a change in the central immunological apparatus has occurred. Some of these experiments—for example, those with the cattle twins—are in essence mutual transplantations of tissues between embryos, in which immunological tolerance develops as the tissues differentiate. Where the adult lymphoid tissues are injected into the fetal or neonatal animal, apparently the tolerance reaction occurs only in the differentiating embryo, since the implant itself will eventually form antibodies against its host.

Initially, the association of the tolerance-inducing antigens with a nuclear fraction from the cells encouraged the speculation that these were involved with the deoxyribonucleic acid (*22*) and that some kind of transductive process might be occurring (*23*). More recent evidence tends to minimize this possibility (*23*). Obviously, proof of a transductive process necessitates genetic markers, and where these markers are present in the blood antigens of cattle twins, Owen (*24*) has emphasized that only the two types of blood group expected from the nature of the cross occur. In this case at least there is no evidence of the free and frequent transduction that would be required for tolerance.

The application of the *Paramecium* serotype transformation analogy to the induction of tolerance follows much the same line as application of the analogy in the F_1 change. Here the environmental shock comes from the foreign antigens of the implant, which would be presumed to change the cytoplasmic state of the differentiating cell from a condition reactive to the foreign antigen to one in

which the mature cell no longer responds by the synthesis of antibodies. The criterion of the change is complementary to that observed in the tumor cell. The change in the tumor cell in the F_1 hybrid is in the relation of its antigens to the hosts' antibodies; the change in the tolerant host is in the cells of its immunological apparatus, which no longer respond to the specific foreign antigens of the kind injected while these cells were maturing.

The apparent critical nature of the stage of differentiation for the induction of tolerance suggests that the realization of the histocompatibility factors may be one of the terminal stages in the differentiation of tissues and places this problem in the general field of embryonic differentiation. Mitchison (*25*) has provided evidence of cell multiplication for antibody-producing cells in the transplants of immunized lymph nodes; it follows, therefore, that the difference between mature and immature cells in these tissues is not simply a matter of the possibility of multiplication. Whether mature lymphoid cells, subjected to foreign substances (antigens or antibodies) under conditions in which they may multiply before the host's defenses come into action, can respond by the establishment of a neutral state (now tolerant of the host antigens) is a matter of conjecture. This is indeed what the tumor cells seem to be doing, both in the F_1 change and in the experiments reported by Koprowski (*26*) in which tumors were injected into fetal, foreign-strain mice. A variety of response from tissue to tissue is perhaps to be expected here.

In summarizing the foregoing sketch, it seems fair to state that neither the phenomena of tumor adaptation nor those of actively acquired tolerance are inconsistent with a scheme derived from the *Paramecium* analysis: constant genetic constitution, responding to triggering environmental stimuli by cytoplasmic states of synthesis, special for each locus. In the *Paramecium* case, the states are in general mutually exclusive; the histocompatibility loci appear to act independently of each other, although the degree to which this is established may perhaps be questioned. In both the instances considered, the process of antibody formation is involved, and in fact the finest discriminations are provided by preimmunization of the hosts, as Amos *et al.* (*27*), the Kleins (*7*), and Snell (*16*) have indicated. Let us now examine the applicability of the serotype transformation analogy to antibody formation.

Antibody Formation

It is not necessary here to review the characteristics of the antibody response; the reader is referred to several recent symposia (*28*), and to the treatment in Burnet's monograph (*29*), for a guide to the relevant literature. The earlier theoretical treatments were dominated by an attempt to understand the chemical mechanisms whereby specific configurations within large molecules could be replicated; the various forms of template hypothesis were the fruit of these endeavors. With the refinement of histochemical techniques and the elaboration of the methodology of cell transfer, the cellular basis of the antibody response is now being emphasized (*30*). The complex of cells in the lymph nodes and in related tissues like the spleen emerges as the protagonist in the response to an antigen. The various accounts agree in distinguishing sharply between the cellular response to an initial exposure to antigen (primary response) and a later exposure (secondary response). By the use of labeled antigens, ample evidence of antigen entry into the nucleus of cells during the primary response has been obtained, but only a few cells are thus affected, and the number of these showing antibody is low. All these cells belong to a special type: the immature plasma cell. The appearance of antibody in the serum follows the differentiation of the plasma cells in the lymph nodes. The dramatic events follow the secondary injection of antigen: Now there are large numbers of plasma cells, in clusters and showing antibody; following this, the elevated serum antibody level characteristic of the secondary response makes its appearance. It thus appears that the first exposure to antigen establishes a mode of differentiation, while the second affords a relatively specific stimulus to proliferation.

It should be recognized here that the older template theories, according to which antibody formation depended on the folding of pre-existing globulin on a pattern provided by the antigen, are now obsolete. New protein is synthesized in the newly proliferated cells, and the more recent theoretical treatments have all recognized that there must be an increase in number of templates as the result of cell proliferation to account for the rich variety of experimental fact. Schweet and Owen (*13*), for example, call for a change in the nuclear heredity (not necessarily genic?) of the antibody-forming cell (deoxyribonucleic acid change), while Burnet (*29*) favors a specifically induced change of a cytoplasmic template system, which he calls a genocopy, associates with the ribonucleic acid containing granules of the cytoplasm and nucleolus, and believes to be capable of replication. Both these treatments avoid the requirement of the earlier theories for the continued presence of antigen in the cell to account for antibody formation after long periods of time. The possibility of antigen persistence is perhaps still not excluded, but this has always strained the bounds of credibility as a general proposition—all the more so with the demonstration that cell proliferation is part of the response to the antigen.

The parallel between Burnet's treatment and the *Paramecium* serotype analysis is evident, once attention is directed to it. But the application demanded is somewhat more complex than that envisaged either by Burnet or by Schweet and Owen. In both of these treatments the secondary response presents a problem, explicitly recognized as unsolved by Burnet. Schweet and Owen have the antigen acting at two sites, first in the formation of a new deoxyribonucleic acid template and subsequently as an inducer, acting to influence synthesis of templates (ribonucleic acid containing) for antibody formation. The influence on the secondary response is deemed to follow from the inducer action. In neither of these is there any inherent reason for the wave of mitosis in the specifically antibody-forming cells.

According to the serotype analogy it would be expected that the antigen would determine the formation of a cytoplasmic state, the nature of which is discussed below, capable of forming its complementary antibody. The problem appears in its clearest form in consideration of the secondary response: Why should the antigen-stimulated cells divide? Is there any basis for assuming that an antigen-antibody type of mechanism can be a stimulus to mitosis?

During the primary response, the mitotic activity is moderate; there seems in fact to be no evidence necessitating any specific high mitotic activity. The cytoplasm of such cells would, on this assumption, contain antibody (and it does, on the evidence of fluorescent antigens). The stem cells of this line are those that multiply in the secondary response. They are stimulated to do so by the presence of antigen. If, as is probable, they contain antibody, they afford the opportunity for the combination of antigen and antibody at the cell surface.

Here it is necessary to recall the distinction between a stimulus to growth by

increase in nuclear and cytoplasmic substance and by the partition of nuclear and cytoplasmic units into separate cells at mitosis. The varied nature of the antigens makes it difficult to conceive that they act to supply the materials for cell growth; it seems unlikely that the stimulus to mitosis is the result of an immediate growth process. The alternative view seems preferable—that the antigen itself acts as an inciter of cell division in the cells capable of forming or already containing the complementary antibody.

This suggestion, while new for the antibody system, has been the basis of attempts to analyze the nature of the stimulus to mitosis in the egg cell at fertilization. For some time, Tyler (*31*), in particular, has investigated the implications of the hypothesis that the egg contains substances complementary to those at the sperm surface, which take part in the fertilization reactions. Quite recently, Perlman (*32*) has shown the presence in the egg of antigens, the antibodies to which are capable of activating the egg—that is, of initiating mitosis in the way postulated for the reaction of antigen with antibody at the surface of the lymphocyte.

It must be supposed here that the stem cells of the plasmacytic line, which contain antibody as a result of the primary response, need only the stimulus afforded by a surface reaction to go into mitosis; and that this stimulus is provided by the membrane change resulting from antigen-antibody combination. The process is the converse of that described for the sea-urchin egg by Perlmann; in that case the antigen is in the cell surface, the antibody coming to it from the environment. But in both cases an antigen-antibody combination occurs at the cell surface to supply a stimulus to mitosis.

The consequences for the intensive nature of the secondary response are apparent. In a lymph node the cells already forming antibody would be stimulated selectively to proliferate, by the newly arriving antigen. Since the specific antibody-forming cytoplasmic state has already been established, the cells so stimulated would increase the response exponentially. Whether, coincident with the specific excitation by antigen, other neighboring cells are also stimulated to a degree is, obviously, a secondary question in this context; the focal point of the problem is, as has been stated, why the specific secondary response should occur.

Further possible parallels exist between the mitotic processes in the egg and those of the plasma cell: Tyler (*31*) has reported the blocking of cleavage by what appear to be massive doses of antibodies to a fertilizing preparation from the jelly layer of the egg. The phenomena of immunological paralysis by massive antigen concentrations [cited in (*21*)] (blockage of proliferation by an extensive antigen-antibody combination at the cell surface) stand in much the same converse relation to this as the selective proliferation of antibody-containing cells does to the activation of the egg.

After the foregoing presentation had been written, J. Lederberg kindly called my attention to the review of Talmage (*33*) and to the note of Burnet (*33*), in which a hypothesis of selective proliferation of antigen-modified cells is developed. Both of these treatments take as the point of departure the combination of antigen with a pre-existing antibody, along the lines of Jerne's natural-selection hypothesis. They differ from the foregoing presentation with respect to the role of the antigen in the primary response; here this response is considered to be in the nature of a change in cell heredity, the latter proliferative response being selective. The treatment presented in this article avoids the awkward assumption that the immunological cell system contains cells synthesizing all possible antibody structures; that of Burnet and Talmage, on the other hand, requires only one function of antigen—namely, its combination with antibody. Experiments on antibody formation in single cells along the lines of those carried out by Nossal and Lederberg (*34*) may serve as a means of distinguishing between these possibilities. With either view, the analogy with the initiation of mitosis in the fertilization process is helpful.

A word may be added anent the problem of self-recognition, so prominent in Burnet's thinking. From the genetic point of view, the loci concerned with self-recognition belong to the histocompatibility group. From the discussion of tolerance already presented, it is evident that the cytoplasmic states conditioned in the cells of the organism's defense systems would be neutral—that is, would form gamma globulins noncomplementary to the antigens present in their embryonic environment. These considerations, however, lead into the field also of growth regulation, discussed in terms of complementary substances by Paul Weiss (*35*) especially, and would take the discussion too far from its base to be dealt with here in detail.

A Possible Chromosomal Basis

In the foregoing discussion the attempt has been made to encompass three quite diverse types of cellular change in mammals in terms of a mechanism for cellular heredity worked out in ciliate Protozoa. Each of these phenomena has at one time or another been thought of in terms of the mechanisms of protein synthesis and their direction by template systems, and in recent times the templates have been identified with the deoxyribonucleic acid molecules found in the chromosomes. Particularly is this true for the antibody-synthesizing systems (*13*), and the suggestion has also been made for the tolerance system (*23*). But an explicit discussion in terms of known mechanisms operating at the chromosomal level, and effective for action on the cytoplasm, has been lacking, and I shall now venture to present one.

The dilemma presents itself clearly in the *Paramecium* case (*11*). The cytoplasmic state, though quasi-autonomous, is nevertheless under nuclear control. Once the pattern is set, the competitive inhibitions postulated for the different postulated cytoplasmic states could possibly account for the autonomy, but the setting of the pattern depends on a specific chromosomal locus. In nucleocytoplasmic terms, the same requirements appear in the other cases also: (i) a definite chromosomal locus must respond to the special stimulus; (ii) the response occurs independently in each chromosome, under most conditions thus far studied; (iii) a variety of alleles is possible at each locus, the loci being genetically complex.

During the past few years, a series of observations on the giant chromosomes of the Diptera have accumulated, which now provide a cytochemical model of chromosome behavior suitable for the activities under discussion. Both in the chironomids (midges), which have been studied in Bauer's laboratory, particularly by Beerman and by Mechelke (*36*), and in the Brazilian gnat *Rhynchosciara* (*37*), structural changes in the chromosomes ("puffs") occur at times and places appointed by the activities of the cells in which they reside. There is here a specific functional response of the genetic system, of the type posited by the immunogenetic reactions under discussion. The "puffs" are formed or regress according to the cell type and according to the cell's stage of development or of function. It may reasonably be supposed that such behavior is not limited to the giant chromosomes—

rather, that they are the extreme case of a general mode of action.

The cytochemical analysis of these puffs is relevant to the discussions of possible changes in the deoxyribonucleic acid of the nucleus. Schweet and Owen had to postulate that a change in the kind of deoxyribonucleic acid is a special property of the antibody-forming system in response to the antigen. But Breuer and Pavan observed a massive increase in Feulgen stainability of the bands concerned with puff formation, which they interpreted as an actual increase of deoxyribonucleic acid in this process. The suggestion was immediately made that here was evidence for the direct quantitative involvement of deoxyribonucleic acid in gene activity, different from its previously postulated function as a constant framework for secondary ribonucleic acid template formation. Proof of the local disproportionate increase in deoxyribonucleic acid has been provided by the work of Rudkin and Corlette (*38*) in my laboratory, in which quantitative measurements of ultraviolet absorption and specific extraction procedures on *Rhynchosciara* chromosomes were used. Similar evidence has been provided for a chironomid, with measurements of Feulgen stainability (*39*). These measurements, together with the tritiated thymidine incorporation studies of Ficq and Pavan (*40*), support the serious consideration of differential deoxyribonucleic acid synthesis as a regular mode of nuclear response. The degree to which this synthesis provides a mechanism for irreversible differentiation has not yet been adequately explored; it is sufficient to recall the fact that the occurrence of irreversible changes in nuclei during embryonic differentiation has been demonstrated by King and Briggs in the frog (*41*), and this must be taken into account in considering the genetics of somatic cells (*42*).

With the synthesis of a special deoxyribonucleic acid in response to a cellular stimulus, the possibility of the direction of the synthesis to a new kind of deoxyribonucleic acid no longer is completely *ad hoc*; the extra deoxyribonucleic acid of the puff permits speculations about changes in composition according to the supply of precursors and by a displacement of its normal protein from the deoxyribonucleic acid, which makes possible a new sequence of bases in the deoxyribonucleic acid chain. However this may be, it is to the ribonucleic acid system that we must look for cytoplasmic states; and here the electron microscope studies are useful. For the dense ribonucleic acid containing granules, which with a complex of double membranes form the endoplasmic reticulum of cells [the framework of the cytoplasm (*43*)], are found in the nucleus also, and Gay (*44*) has shown a relationship in the salivary-gland nuclei of *Drosophila* between structures derived from particular loci on the chromosome and the endoplasmic reticulum of the cytoplasm. In mammalian cells, it appears that the nuclear membrane is part of the endoplasmic reticulum (*45*), affording thus perhaps a direct line of communication between the nucleus and the exterior of the cell. The point of this exquisite detail is obvious: it provides the possibility for reactions of specific loci on the chromosome to special groups of environmental changes. There is no need to elaborate such possibilities; they provide a basis for the specific reactivities that have been discussed, whose experimental analysis is still in its infancy. The integration of this structural analysis with the concepts of enzyme induction (*46*), from the permeases at the cell surface to the synthesis of the specific deoxyribonucleic acid in the nucleus, is a challenging problem.

This discussion, by one more familiar with chromosomes than with immunological reactions, only serves a purpose if it places in cytogenetic perspective the problems arising in the consideration of transplantation specificity, actively acquired tolerance, and antibody synthesis as cellular phenotypes. If the analogies made with the ciliate work are valid, the repercussions on other related phenomena need to be examined—for example, the relation between chromosome balance and transplantation specificity (Hauschka and Amos) or the attempt to study mutational changes at the H-2 locus in heterozygous tumors, made by Klein (*47*), which finds a challenging parallel in the instability of certain serotypes in heterozygotes, noted by Sonneborn *et al.* (*48*). We are now in the difficult terrain between mutation (in which for the moment such phenomena as transduction may be included) and the types of nuclear differentiation just beginning to be explored, and already mentioned. Desirable experiments are many, focusing on the exploration of antigenic changes in cells cultured under defined conditions, excluding selection effects, and exploring the range of phenotypes possible for a given genotype. These experiments are easier to list than to carry out but at the present state of the art not impossible (*49*).

Summary

The paradoxical features of transplantation specificity—its strict genetic control in transfers of tissue from strain to strain as compared with its malleability on tissue passage in foreign immunological environments where the host does not reject the implant (F_1 hybrid passage, tolerance actively acquired by immature hosts, and so on)—present a challenge to genetic interpretation. The attempt is made in this article to show parallels between this behavior and such changes as the transformation of serotypes in *Paramecium,* in which the activity of genetic units becomes fixed as a cytoplasmic state—a cellular heredity persistent under specified environmental conditions but capable of change to an alternative state—while the genetic structure of the cell remains constant. The reactions appear to differ from those in the *Paramecium* case in that the diverse loci control a mosaic of different specificities, which change relatively independently of each other, in contrast to mutual exclusion of cytoplasmic states influenced by the different loci in *Paramecium.*

The process of antibody formation is considered as a change in cellular phenotype from the same point of view. The primary response in the stem cells of the lymphoid tissues is interpretable as the establishment of a new cytoplasmic state in response to a nuclear stimulus by the foreign antigen. For the secondary response, the suggestion is made that a reaction of antigen with cellular antibody at the surface of stem cells exhibiting the primary response serves as the stimulus for specific proliferation of antibody-forming clones of cells. A parallel is drawn with the fertilization reaction, specifically with regard to the initiation of cleavage in eggs by antisera to them.

Finally, a general chromosomal mechanism is sought for these phenomena, on the basis of activities of specific chromosome regions in response to special developmental stimuli, such as the disproportionate local synthesis of deoxyribonucleic acid demonstrated in the giant chromosomes of the Diptera. By a correlation of such activities with the nucleocytoplasmic system of ribonucleic acid granules on membranes, a possible mechanism appears for the formation, in response to environmental stimuli, of cytoplasmic states which might supply the persistent pattern required for this type of cell heredity. The analogies made, it is believed, provide a framework for the design of test experiments.

References and Notes

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CURRENT PROBLEMS IN RESEARCH

Thermoelectricity at Very Low Temperatures

Kelvin's discovery may be the key today to electron transport problems.

D. K. C. MacDonald

The experimental discovery of thermoelectricity dates from early in the last century. The Seebeck potential, or thermoelectric force, is the voltage produced in a circuit of two dissimilar elements when one junction is heated relative to the other (Fig. 1). The Peltier heat is the component of heat evolved or absorbed at the junctions per unit time when unit current flows in a circuit. It was William Thomson (Lord Kelvin), however, who in 1854 essayed a thermodynamic analysis of these effects (*1*), recognizing that they must be interrelated, and who was then led to make the remarkable prediction that a third effect (known today as the Thomson heat) must exist in a single conductor when a current flows through it and the conductor is in a temperature gradient. The Thomson heat is reversible in the sense that a component of heat is evolved or absorbed, depending on the relative direction of the electric current and temperature gradient. After his theoretical prediction, Thomson then went on to show the existence of this effect by a painstaking series of experiments. The definition of the Thomson coefficient μ is given by the following equation:

$$Q = -\mu J_x \frac{dT}{dx} + \frac{J_x^2}{\sigma} \qquad (1)$$

where Q is the heat evolved per unit volume per unit time in a conductor; J_x is the current density; dT/dx is the temperature gradient; and σ is the electrical conductivity.

The second term in Eq. 1 corresponds to the *irreversible* Joule heating, and it

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