plications regarding the nature of gene action and the genetic control of differentiation.

Recently, Brink and Nilan, in a reinvestigation of varigated pericarp, reported data which suggest that mutation at the *P* locus involves a mechanism similar to that of *Ac-Ds,* and Peterson from his analysis of a mutable pale green locus concluded that an *Ac*-like factor was operating.

As was true in the study of genes, information on the nature of the cytoplasm and its particulate components can come only when heritable modifications occur whose characteristics can be compared with the normal condition. The chloroplast is a particulate cytoplasmic component that has a high degree of autonomy. Although morphological evidence for chloroplast continuity may be inconclusive, there is good evidence for genetic continuity from breeding experiments.

Plastid mutations not only occur spontaneously but can be induced by the action of specific nuclear genes, as has been demonstrated in two cases in maize. A second type of cytoplasmic change is that responsible for pollen abortion or male sterility. This kind of cytoplasmic mutation has occurred a number of times and is currently of great interest to the hybrid-seed producer, since its utilization promises to make unnecessary the costly and tedious task of detasseling. It is likely that the cytoplasmic condition responsible for male sterility arises from the mutation of a particulate cytoplasmic factor, but this has not yet been fully demonstrated. That interaction of genic and cytoplasmic factors is involved in the expression of the male sterile phenotype became apparent when it was found that certain races carried specific genes that were able to suppress the male sterility factors in the cytoplasm, while other strains had genes that were quite ineffective in this respect. Jones found that some lines had a single dominant gene that would restore pollen fertility. The dissimilar effect of specific fertility-restoring genes on different sources of sterile cytoplasm suggests that these cytoplasmic mutations are not all identical. A complex situation was encountered in the male sterile condition analyzed by Schwartz, where pollen abortion was produced only when a specific kind of cytoplasm was combined with a dominent gene for male sterility and with the recessive allele of a suppressor locus.

Although the amount of work on cytoplasmic inheritance has been negligible compared with that on genes and chromosomes, it may be confidently expected that significant advances will be forthcoming in the future.

Note

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Nuclear Function and Mitosis

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WHEN a cell goes into mitotic division, the state of the nucleus is altered in every visible respect from that which is observed in the cell when it is not dividing (interphase). In interphase, the nucleus is seen as a distinct body, encased in a membrane. The chromosomes exist in a diffuse condition and are seldom resolvable as distinct threads. Often one or more nucleoli are present. In the preparatory stages of mitosis, the nuclear membrane and the nucleoli disappear, and the chromosomes condense into compact bodies of high density. The relationship between these states of the nuclear apparatus and the functions of the nucleus is a question of some importance. The nucleus performs two classes of functions. The reproductive functions include the processes that lead to the production of a new set of chromosomes identical with the original set, guaranteeing that the daughter cells of a division will obtain a complete representation of the genetic material present in the mother cell. It now seems to be established with fair certainty that the syntheses involved in chromosome reproduction take place during interphase and not during the mitotic process itself (*1*).

The class of nuclear functions that may be termed "physiological" includes all nuclear activities that are necessary for the long-term survival of the cell when it is not reproducing. It is reasonably certain that the interphase nucleus performs such functions (*2*).

The question to be considered is whether the physiological activities of the nucleus depend on its being in interphase condition, or whether they can be carried out during division when the chromosomes are condensed and are not separated from the cytoplasm by a membrane.

An experimental attack on this question poses two requirements: (i) an adequate supply of interphase cells and dividing cells for comparison; (ii) a criterion for evaluating the physiological activity of the nucleus. Both of these requirements may be met by choosing the familiar ameba, *Amoeba proteus,* as experimental material.

It has recently been found by T. W. James (*3*), in this laboratory, that members of clones of *A. proteus* will divide quite synchronously if the temperature is cycled. In these experiments, a daily cycle of 12 hr at 18°C and 12 hr at 26°C was employed. At appropriate times (shortly after the beginning of the high-

temperature period), a considerable proportion of the cell population was observed to have entered mitosis, and these could be identified and recovered from the cultures. There was no difficulty in obtaining sufficient numbers of dividing cells for the experiments (*4*) described in this paper. Details of the culture system will be published elsewhere, but it should be noted that a similar principle has been applied recently by Scherbaum and Zeuthen (*5*) in obtaining synchronously dividing populations of *Tetrahymena*.

The criterion of nuclear function applied in this investigation was the magnitude of the incorporation of phosphate, measured as P^{32}, into cell constituents. The effect of the nucleus on this group of processes was discovered by Mazia and Hirshfield (*6*) and has been confirmed and extended by Brachet and coworkers (*7*). It has been shown that within the first hours after removal of the nucleus from *A. proteus*, the amount of P^{32} incorporated in a given time has dropped to 30 to 50 percent of that taken up by normal cells under the same conditions. Other studies on *A. proteus* have shown that, during the same time interval, the respiration (*8*), the level of a number of enzymes (*9–11*), the permeability to water (*12*), and the uptake of neutral red (*13*) seem to be indifferent to the presence or absence of the nucleus. Thus, while the significance of the relationship between the nucleus and phosphate incorporation is not understood, it will serve the purpose of a criterion of nuclear activity. The question we ask is simply: Does the phosphate incorporation of the ameba during division resemble that of an interphase ameba with a nucleus or of an interphase ameba without a nucleus?

The experimental procedure was as follows. The control sample consisted of 25 nondividing amebas isolated from a population that was in the latter half of the interphase period, when the cells had passed the stage of maximum growth. An equal number of amebas at "prophase" was selected for comparison with the nondividing group. The criterions of "prophase" were the rounding up of the organism, the absence of a distinct nucleus, and the absence of a division furrow. The two groups of amebas were placed in an inorganic medium, free from food organisms, to which $P^{32}O^4$ had been added as sodium salts to a final activity of 20 μc/ml. After 20 min exposure at 21°C, the cells were washed repeatedly with nonradioactive medium until the fluid showed no detectable radioactivity. The amebas were then mounted in sample pans, dried, and assayed for P^{32} with the aid of a windowless Geiger counter.

The results are given in Table 1. It will be noted that a number of the cells divided during the experiment. This fact shows, on the one hand, that the exposure to the radioactive medium was harmless but, on the other hand, it signifies that some of the dividing amebas were probably at a later stage than others when selected.

It is evident that the P^{32} incorporation by the dividing amebas is only about one-half of that of the interphase amebas. In a general way, therefore, it may be concluded that when an ameba enters division, the P^{32} uptake is more comparable to that of an interphase cell without a nucleus than to that of one with a nucleus. If the level of P^{32} incorporation is in fact a criterion of the physiological functioning of the nucleus as defined earlier in this paper, it is to be concluded that the nuclear functions are absent or survive at a very depressed level during mitosis.

There is an apparent quantitative difference between the relative levels of P^{32} incorporation in dividing cells and in cells without nuclei when both are compared with nucleated interphase cells. Entry into division lowers the level by a factor of 2, while the earlier data of Mazia and Hirshfield indicated that the removal of the nucleus lowered it by a factor of 3. The discrepancy could signify a residue of nuclear activity during division. However, the values for the dividing cells could easily be too high, because the length of exposure to the isotope was such that some of the dividing cells may have reconstituted their nuclei during the experiment. The data on the number of cells actually observed to complete their division before the end of the experiment suggest that this source of error exists. Likewise, the control levels could have been low compared with those in the earlier experiments, because only "late" interphase cells were chosen in the present case.

The depression of an activity of the cell during division is not in itself unexpected. Zeuthen (*14*) describes experiments on dividing sea urchin eggs which show a brief depression of phosphate uptake during each division. Zeuthen (*14*) also observes, in *Tetrahymena*, that the rate of respiration, which increases in a linear fashion during interphase, levels off during division.

The present work tends to relate cases of depressed cell activity during division to the absence of nuclear functions. If our results permit generalization, it is predicted that any nucleus-dependent operation of the cell will drop to a lower level during division and, conversely, that such a decline will be indicative of participation of the nucleus. In these terms, Zeuthen's observation that respiration in *Tetrahymena* does not decline during division, but that the synthesis of respiratory machinery ceases, is quite reasonable in view of the postulated role of the nucleus in specific biosyntheses (*2*).

Table 1. Comparison of P^{32} incorporation into *Amoeba proteus* at interphase and during mitosis.

Expt. No.	Counts/min per cell		Ratio (interphase/dividing)	Divisions completed during exposure
	Interphase	Dividing		
1	2.3	1.3	1.8	4 of 23
2	2.1	1.2	1.7	1 of 20
3	2.7	1.7	1.6	6 of 25
4	2.7	1.5	1.8	7 of 25
5	2.0	1.1	1.8	4 of 25
6	1.9	1.0	1.9	8 of 25
Avg.	2.3	1.3	1.8	

It seems probable that physiological activities of the nucleus on which the cell depends for long-term normal function depend somehow on the conditions existing in the interphase nucleus. It is not difficult to propose reasons why this should be the case. First, it is quite conceivable that the condensed condition of the chromosomes during division is incompatible with their functioning. Perhaps it would be more proper to say that the highly dispersed condition during interphase is more compatible with the essentially two-dimensional "template" functions that are currently the subject of so much interest (*15, 16*). Second, the hypothesis that the specific functions of the chromosomes depend on their being provided with an appropriate intranuclear environment (*17*) would lead one to predict important alterations in chromosomal function during division, when the nuclear membrane is altered or absent. Finally, and on a somewhat different level, the disappearance of the nucleolus during division should be noted. There is now excellent evidence that the nucleolus is an important center of nuclear function (*18*). If this structure, as has been proposed, mediates in the action of the chromosome on the cytoplasm, it is only to be expected that such action will not be observed in the dividing cell where it is absent.

The present experiments obviously do not favor any one of these alternatives or even limit the possibilities to these three. They do, however, tend to prove what has been suspected: that the contributions of the nucleus to cell function depend on the conditions characteristic of its state when it is not dividing and that the condensed thread so useful to the cytologist is a very poor model for a working chromosome.

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Fungitoxicity of Ethylenethiourea Derivatives

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THE ability to predict fungitoxicity of compounds has been, and continues to be, an elusive but much desired goal. Clues to structural changes that appear to increase activity are many. It is only seldom, however, that a suggested change, based on rationale, results in the creation of a fungitoxic molecule from a configuration that is completely inactive. This is a report of one such case (*1*).

The inactive compound chosen for modification was ethylenethiourea. This heterocylic nitrogen compound is similar in structure to the imidazolines and pyrazoles, derivatives of which have been found to be very fungitoxic (*2, 3*). In addition, ethylenethiourea contains a thioketone group that is present in many fungicides, particularly the derivatives of dithiocarbamic acid. Ethylenethiourea, therefore, appears to have many of the requirements of a fungitoxic compound; yet, it is inactive when tested for fungitoxicity by the depression slide technique (*4*).

A second reason for interest in ethylenethiourea is that it has been identified (*5*) as one of the break-down products of nabam (disodium ethylenebisdithiocarbamate). Because of its lack of fungitoxicity, it was thought, however, that this compound could not be directly involved in the fungitoxicity of nabam. Could it be, however, that ethylenethiourea is unable to penetrate through the semipermeable membrane of the spore to the site of action? It is possible that nabam, or one of its intermediate break-down products, penetrates readily, decomposing inside to release ethylenethiourea *in situ*. If this were so, then ethylenethiourea itself could exert fungitoxicity if it were changed in such a way as to increase penetration to the site of action within the spore.

One of the simpler methods of increasing penetration is the addition of a hydrocarbon substituent to the molecule (*6*). This possibility was examined by measuring the fungitoxicity of some alkyl-substituted derivatives of ethylenethiourea (*7*). These were propylenethiourea, N,N′-di-n-butylethylenethiourea, N-n-octylethylenethiourea, N-t-octylethylenethiourea,