AN individual paramecium can synthesize many serologically distinct surface antigens, each of which is specified by a separate locus. The environment, acting through the cytoplasm, selects which of these ciliary proteins is to be expressed. All of the remaining immobilization antigens are excluded from expression (Beale 1957). In a constant environment each serotype can be passed on and maintained by a cell’s descendants.

Recent observations suggest that cells can assume a different character when cultivated in low densities and numbers compared with comparable cells grown en masse. From a clone of paramecia heterozygous for a particular serotype, antigen can be extracted that appears homogeneous. Only one kind of antigen is detected per clone, even from clones composed of cells capable of synthesizing several kinds of hybrid antigens (Finger, Onorato, Heller and Wilcox 1966). In a separate series of observations small numbers of cells that were often highly unstable in retaining a particular antigenic type in depression slides seemed more stable when the same clones were grown in tubes or flasks. It seemed reasonable to suppose that a substance was given off and taken up by other cells. In high enough concentration the material ingested would have an effect on the kind of antigen made by the recipient cells. In this manner the entire population would tend to become uniform in phenotype.

The significance of the proposed mechanism lay in its possible application to other biological phenomena associated with cell to cell influences, such as induction during development, differentiation, tissue formation, etc. Thus the phenomenon was thought to have a possible broad relevance and has been examined intensively.

MATERIALS AND METHODS

Stocks and culture: Stock 7, syngen 2, of Paramecium aurelia was used exclusively. Cultures were maintained at 17°C in a Cerophyl medium previously described (Finger 1957) in either 16 × 150 mm culture tubes or 21 Erlenmeyer flasks. Subclones isolated from a parent culture were generally kept at room temperature in depression slides stacked inside moist chambers (Sonneborn 1950).

Antisera collection and determination of serotypes: Sera were prepared against purified immobilization antigen incorporated in Freund’s adjuvant using the footpad route of injection in rabbits (Leskowitz and Waksman 1960). Sera were stored in a deep freezer without preservative.
The serotype of a culture was determined by removing samples and separately exposing each to dilute antisera specifically directed against C, G and X purified antigens.

Antigen purification and detection in vitro: Antigen was extracted from cultures by an ammonium sulfate salting out procedure (PREER 1959) and assayed by the gel diffusion method of PREER (1956). In this method a disc of antigen-antibody precipitate forms in an agar column contained in a small tube. The position of the disc is a measure of the concentration of antigen.

Tests for effects of cell-free medium: The following procedure was followed to determine whether a substance capable of affecting serotype expression is released into the medium in which cells had been swimming (Figure 1). Subclones originating from a single paramecium were transferred from depression slides to test tubes and then finally to 21 flasks filled with about 1.5 l of Cerophyl medium. As soon as the culture fluid has been cleared of bacteria by the feeding paramecia, the cells were concentrated by centrifugation in a DeLaval Porto Lab centrifuge. In the first experiments, 300 ml of the supernatant were set aside for lyophilization, and the remainder was further divided into three or more aliquots. One quarter of the precipitate of live paramecia (ca. 10⁶ cells) was recentrifuged and the packed pellet frozen, to be extracted later for antigen. The remaining paramecia were removed from the rotor and added to three

![Figure 1](image-url)
flasks: one 30 ml aliquot was suspended in 300 ml of fresh culture fluid; a second portion resuspended in 300 ml of its own bacteria-exhausted culture fluid; and the remaining 30 ml aliquot, the experimental, was suspended in the supernatant derived from a different clone. In later experiments these volumes were reduced 100-fold without noticeably affecting the outcome. In some experiments the precipitates were divided into as many as six aliquots involving an equal number of supernatants.

Paramecia mixed with either fresh culture fluid or supernatant were allowed to stand at room temperature overnight in small stoppered culture tubes. To determine the proportion of cells that had been induced to transform, a single cell was isolated from each mixture into each of 60 depressions and after 5 days growth in fresh medium samples of each of the derived 60 subclones were tested for antigenic type. Room temperature (about 20°C) was selected for sustained growth because all three of the most frequently occurring antigenic types, C, G and X, could be maintained at this temperature when continuously in contact with their own supernatants; only occasionally did other serotypes arise. If any animals within a subclone reacted to any of the sera, the subclone was recorded as having cells of that serotype.

The original flask of animals was tested for serotype prior to treatment by testing three aliquots totaling 300 to 500 paramecia. Only flasks 98–100% pure for a serotype were used in these experiments.

RESULTS

Comparison of heterologous and homologous mixtures: Figure 2 illustrates the results obtained with ten of the recipient clones, of which seven were of the C serotype, three of the G serotype. Striking differences in responses were observed when the original medium (isologous culture fluid) was replaced by heterologous culture fluid (i.e. the supernatant from a culture with a serotype different from that of the recipient culture) as contrasted with homologous culture fluid (medium from an other culture of the same serotype as the recipient).

All of these results were from experiments in which recipients were sufficiently unstable to respond to change of culture fluid. The criterion for stability was whether or not a clone's serotype remained unchanged at 95 to 100% of the original serotype when its growth medium was replaced with fresh fluid. The reliability of this standard was tested as follows: prior to separation of the cells from culture fluid, 120 isolates were made into fresh culture fluid in depression slides. Sixty were placed at a temperature favoring the expression of C (17°C) and 60 at a temperature favorable for G (24°C). After about eight fissions samples were tested for serotype. All those clones which had been classified as stable on the basis of their reactions after growth overnight in fresh and exhausted culture fluid at room temperature provided subclones which retained their original serotypes in these more stringent tests. Unstable clones tended to yield a mixture of subclones. Thus identity of behavior in fresh and original culture fluid and maintenance of the initial serotype in at least 95% of subclones as criteria for stability seem justified.

In all, 58 clones have been analyzed involving cross-mixing of 197 precipitates and supernatants. Of the recipients, 25 were stable, with the remainder being of varying degrees of instability. Almost 90% of the stable clones were unaffected by the addition of media from a total of 67 donors of the same or of a different serotype. The remaining description deals exclusively with the responses of the unstable recipients. Table 1 summarizes the effect of replacing the original
medium with heterologous culture fluid. Among those recipient clones affected by the added supernatant, the majority showed a decrease in the percent of subclones expressing the original serotype. (The serotype of a culture was considered to be affected when the treated subclones differed from the isologous mixture by at least 5%). About four fifths of the recipients were about equally divided among G or C clones. Both serotypes yielded similar results.

When the heterologous mixtures are subdivided according to the stability characteristics of the donor cultures, although there is no significant difference between stable and unstable donors (using a 2 x 3 contingency test) a distinction may be indicated. Of the 17 donors of the culture fluid from stable cultures, 70% (12) induced transformation. In contrast, only 42% (13/31) of the supernatants from unstable donors were effective in inducing transformation. More often than not, the recipient clones were stabilized and tended to retain or increase the number of subclones expressing the original serotype. The \( x^2 \)'s calculated to see whether the differences between any two sets of data were larger than would be...
TABLE 1

* Heterologous transformation of paramecia serotypes by addition of cell-free culture fluid (supernatant)

<table>
<thead>
<tr>
<th>Source of supernatant</th>
<th>Response of treated clones</th>
<th>Decrease of initial serotype</th>
<th>Increase of initial serotype</th>
<th>No change</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterologous*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stable clones</td>
<td>12</td>
<td>2</td>
<td>3</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Unstable clones</td>
<td>13</td>
<td>4</td>
<td>14</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>6</td>
<td>17</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Homologous†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stable clones</td>
<td>2</td>
<td>9</td>
<td>7</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Unstable clones</td>
<td>6</td>
<td>9</td>
<td>14</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>18</td>
<td>21</td>
<td>47</td>
<td></td>
</tr>
</tbody>
</table>

* C cells + G supernatant; G cells + C supernatant.
† C cells + C supernatant; G cells + G supernatant.

A parental clone represents one experiment in which 60 subclones were isolated from a single treated clone. About 40 to 50 of these subclones were tested for the presence of cells of C, G or X serotypes.

Expected by chance on a null hypothesis are presented in Table 1. Interesting enough, when examined from the point of view of the donor's serotype, all of the stable donors that elicited a decrease in recipient serotype caused a concomitant increase in subclones of the donor's serotype. In contrast, fewer than half of the unstable donors that induced transformation caused the recipients to assume the serotype of the donor.

Especially instructive is an examination of the relationship between the degree of instability of the donor and recipient and the effect of added medium. Forty-one unstable clones of the same serotype were selected on the basis of differences in the extent of their instability. These clones were mixed in pairwise combination, i.e. medium from one clone was mixed with cells of a second clone and vice-versa. In seven such mixtures there was no difference between the original and the substituted medium. In eight cases, medium from a clone more unstable than the recipient increased the proportion of subclones with transformed cells, or when from a more stable clone decreased the proportion. In 26 mixtures the proportion was either decreased with medium from a less stable clone or increased with medium from a more stable clone. Thus the tendency for a clone's medium to stimulate transformation is correlated with the degree of instability of the clone. If a clone is more stable than a recipient, it will generally increase the frequency of the original serotype. Conversely, a recipient clone will be rendered more unstable by replacing its own medium with medium from an even more unstable sister clone.

Although most of the media (26 of 41) followed the above pattern, the exceptions are sufficiently numerous to suggest that the degree of instability of a clone, while of overriding importance, is probably only one of several factors determining the response of a cell to added medium.
Summarizing, then, these experiments show that: (a) recipient cells are susceptible to transformation of antigenic type only if they are initially unstable; (b) recipient cells are most often induced to transform when the source of added medium is a heterologous serotype; (c) media from stable donors may be more effective than media from unstable donors in inducing transformation; and (d) generally a clone is induced to transform by medium from a culture more unstable than itself and is stabilized by media from donors that are more stable.

**Antigen assay in culture fluid:** These findings could be explained within the framework of a working hypothesis put forward to account for other serotype phenomena. According to this hypothesis the end product of the gene's activity, the ciliary antigen, might stimulate additional synthesis of the antigen via a positive feedback mechanism. Applied to the above instances, the relative uniformity of a mass of cells would be a consequence of antigen being transferred from cell to cell.

If the proposed feedback mechanism applies here, there ought to be present in supernatants antigen which is responsible for the transformations noted. Those supernatants which have little or no effect on susceptible clones should contain little antigen. Furthermore, the kind of antigen present presumably would be of the same serotype as that induced.

The serotype of donors might be expected to be a sufficient guide to the antigen to be found in the supernatant. However, it has been shown previously that the antigen on the surface of a cell need not be the only one synthesized (FINGER, HELLER and GREEN 1962; SEED, SHAVER, FINGER and HELLER 1964). Therefore, antigen was extracted both from the frozen cells and from the lyophilized supernatant. Antigen was purified by the method of PREER (1959) and concentrations were estimated by gel diffusion analysis in PREER tubes (1956). As can be seen from Table 2, antigen may be found in the supernatant. The distribution tends to parallel the antigenic content of the original cells. Also each clone possesses several antigens even though initially every population was uniformly of one immobilization type.

The presence of antigen in the supernatant is, in itself, not proof of the feedback hypothesis. Indeed, the gel diffusion studies show that the kind of antigen

<table>
<thead>
<tr>
<th>Clone No</th>
<th>Serotype</th>
<th>Antigens in cells</th>
<th>Antigens in fluid</th>
<th>Serotypes induced*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>G</td>
<td>C ++ + +</td>
<td>C + -</td>
<td>C, X</td>
</tr>
<tr>
<td>6</td>
<td>G</td>
<td>lost</td>
<td>- - -</td>
<td>G, X</td>
</tr>
<tr>
<td>5</td>
<td>Z</td>
<td>- + + + + +</td>
<td>- - -</td>
<td>Z</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>+++++ - + + + +</td>
<td>+ + + - + + + +</td>
<td>C, G, X</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td>+ + + + + + + +</td>
<td>+ - + + - + + + +</td>
<td>X, C</td>
</tr>
</tbody>
</table>

Relative antigen concentrations (indicated by + 's) were determined by the position of an antigen-antibody precipitate formed in Preer tubes. The greater the antigen concentration, the further down the agar column was a band observed.

* The first serotype listed is that induced with the greatest frequency. The distribution of serotypes of recipient cells resuspended in their own culture fluid was used as controls in determining the effectiveness of the supernatants (fluids).
in the supernatant is *not* correlated with the transforming activity of the supernatant. In fact, several supernatants that elicit transformation had no detectable antigen.

The presence of antigen in the supernatant suggests that the entire phenomenon of cell to cell interaction may be due to the presence of contaminating, living paramecia in the culture fluid added to recipient clones. However, this possibility is ruled out by several observations: (1) Stable clones do not become unstable by the addition of supernatant from unstable clones. (2) The antigens isolated from donor cells were only occasionally those present in abundance in the supernatant. (3) Supernatants on the average had fewer than one cell per ml. Alternatively it may be that cells are broken up in handling and the contents that are released into the medium provide the active substance in culture fluid. Population counts made prior to and following concentration of donor cells speak against this suggestion. In many instances fewer than 4% of the cells were destroyed or lost. Extracts from a considerably larger population of cells were later shown to have little effect on recipients.

Although an explanation for the influence of medium based on feedback via release of antigen is deemed very unlikely, similar feedback mechanisms, involving other substances, of course are not ruled out.

**DISCUSSION**

These experiments demonstrate that transformation from one paramecium antigenic type to another can be induced by the cell-free culture fluid in which paramecia of the second type had been growing. Since the donor and recipient cells were genetically identical, this is an example of induced gene expression, perhaps by the action of one cell's products on another.

It may be difficult to identify the agent(s) responsible for the transforming effect of medium. Numerous factors, such as temperature, pH, cultural conditions, nuclear reorganizations (autogamy and conjugation), etc. are known to exert marked influences on the serotypes manifested by a clone (BEALE 1957). Furthermore, it cannot be stated that the substances in the medium originated from the paramecia, especially since the strains used were not cultured axenically.

In spite of its magnitude, the task of identifying the factors in the medium is certainly worth pursuing. The nature of the agent inducing or repressing antigen synthesis would be of importance in assessing the applicability of this transfer phenomenon to other systems and organisms.

These studies were supported by Grant GM 12017-06 from the Public Health Service.

**SUMMARY**

Transformation from one paramecium antigenic type to another can be induced by the cell-free culture fluid in which paramecia of the second type had been growing. The possibility that this phenomenon is a result of feedback by antigen released into the medium is considered unlikely. Since the donor and
recipient cells were genetically identical, this is an example of the regulation of the expression of specific genes, by perhaps the action of one cell's products on another.

**LITERATURE CITED**


