cytochrome *c* per ml was reduced with hydrogen gas in the presence of a small amount of palladium black until the cytochrome *c* appears to be 80–90 per cent reduced. The excess hydrogen was removed by sweeping the solution with nitrogen. The solution was filtered, allowed to stand for several hours in air at room temperature, and stored at 0°C as stock solution.

*Rate measurements:* The reaction was followed by observing the change in optical density at 550 mμ by means of a Cary Recording Spectrophotometer. All measurements were made in 0.1 F Tris buffer (pH 7.7) containing 0.0005 mole of the connecting ligand per liter of the solution at 22 ± 3°C. The total cytochrome *c* concentration was determined by reducing the solution with a slight excess of $Na_2S_2O_4$ and calculating from the optical densities (O.D.) of the reduced solution by means of the expression

$$[\text{ferrocytochrome } c] = \frac{(\text{O.D.})_{550\ m\mu} - (\text{O.D.})_{530\ m\mu}}{2.0 \times 10^4}.$$

The cytochrome *c* solutions are approximately 70 per cent reduced at the beginning of the rate measurements.

A blank experiment with cytochrome *c* dissolved in buffer solution containing 0.0005 mole/liter of the connecting ligand but without added catalyst was made concurrently with each experiment. These blank rates were subtracted from the observed rates of catalyzed reactions before the computation of the rate constants.

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† Contribution No. 1618 from Sterling Chemistry Laboratory, Yale University, New Haven, Connecticut.

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## *TISSUE CULTURE POPULATIONS AND THEIR RELATION TO THE TISSUE OF ORIGIN**

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*Introduction.*—Cultures of continuously propagated mammalian cells are usually devoid of properties specific to the tissue of origin. In fact, cultures derived from different tissues bear a remarkable similarity to one another. Among the many properties common to tissue cultures derived from a single species, irrespective of their tissue of origin, or whether malignant or normal, are antigenic specifi-

city,[1, 2] a requirement for certain amino acids nonessential for whole animal nutrition,[3] enzyme levels,[4] pattern of carbohydrate metabolism,[5] and sensitivity to chemotherapeutic agents.[6, 7] The various explanations proposed for this phenomenon can be summarized and simplified in the form of alternative hypotheses. According to the first hypothesis, tissue culture populations are descendants of the parenchymal cells of the tissue of origin, but in the process of multiplication these cells lose their differentiated properties and gain new properties in response to the dictates of the *in vitro* environment. In compliance with current usage, this hypothesis will be referred to as the dedifferentiation hypothesis. According to the second hypothesis, tissue culture populations are not derived from the parenchymal cells of the tissue of origin, but are descended from a ubiquitous minority cell type of the inoculum. The latter will be referred to as the selection hypothesis. In an attempt to distinguish between these two possibilities the following experimenta plan was adopted. Cultures were initiated from liver of day-old rats. After a brief period of growth, the nonviable portion of the inoculum was separated from the growing population. The attached and stretched cells (i.e., the growing cells) were examined for liver specific properties. According to the dedifferentiation hypothesis, one would expect a gradual and selective loss of liver specific properties rather than a simultaneous and complete loss of all liver properties. According to the second hypothesis, since the growing population is descended from cells not possessing liver specific properties, there should be a complete absence of liver specific properties at all times. Finally, liver inocula were pretreated with antisera specific against liver and antisera specific against tissue cultures and cultured in the absence of antisera. According to the dedifferentiation hypothesis, pretreatment with antiliver antiserum should destroy those cells which generate the ultimate tissue culture population and, as a consequence, completely inhibit subsequent growth. On the other hand, the selection hypothesis predicts that pretreatment of liver inocula with antitissue culture antiserum should, while leaving the liver parenchyma intact, destroy those cells destined to give rise to the final population. The markers used in these studies were liver antigenicity, ornithine transcarbamylase activity, and serum albumin content.

*Experimental Methods and Materials.—Media:* Eagle's Hela Medium,[8] with the addition of serine at 0.2 mg/ml and inositol at 0.4 mg/ml, was employed as the standard synthetic medium. The culture medium consisted of synthetic medium plus 15 per cent horse serum and 5 per cent fetal calf serum. All cultures were grown in 60 × 15 mm Petri dishes and incubated at 37°C in an atmosphere of 5 per cent $CO_2$ and 95 per cent air.

*Inocula:* One-day-old Sprague Dawley rats were sacrificed by decapitation and blood removed by perfusion of the whole body with saline injected into the left ventricle. The liver was then removed and sliced with a McIlwain tissue microtome in two directions at a setting of 0.2 mm. The resulting inocula had a distribution of sizes ranging from single cells to pieces of tissue 1.0 mm in diameter. When a suspension of single cells was required, the setting on the microtome was reduced to 0.05 mm, the liver sliced and suspended in neutralized synthetic medium, and the suspension forced through a 25 gauge needle with a syringe. To remove cellular debris both types of inocula were washed in the synthetic medium neutralized to pH 7.0. Single cell suspensions of adult liver were produced in a similar

manner except that the animals were perfused by injection of saline through the hepatic portal vein and drainage through the severed inferior vena cava.

Ornithine transcarbamylase assays were performed according to the method of M. E. Jones *et al.*[9] Serum albumin was assayed by a sensitive micro complement fixation technique developed by E. Wasserman and L. Levine.[10] Protein was determined by the method of Lowry *et al.*[11] with crystalline bovine serum albumin as standard.

*Antisera:* Rabbit antisera were prepared by the following immunization schedule. Each antigen at a level of $5 \times 10^6$ cells/ml was thoroughly washed in saline, suspended in Freund's adjuvant, and injected in two subcutaneous and two intramuscular depots. This was repeated after a three-week interval. One month later the rabbits received an intravenous injection of $10^6$ cells in saline, and were bled after seven to ten days. Subsequently, the animals received intravenous booster injections at monthly intervals and serum was taken after a week. To obtain anti-rat-liver antisera, purified single cell suspensions of adult rat liver were used. For production of antisera to tissue cultures, cells were harvested by trypsinization of tissue cultures, and the trypsin removed by washing.

Absorption of antisera was performed either with suspensions of adult rat liver cells or with a minced preparation of hepatectomized day-old rats. Both preparations were thoroughly washed with saline prior to use. For each 10 ml of antisera, 1.0 gm. of tissue was added, and the resulting suspension incubated with frequent agitation for 30 min at room temperature. The absorption material was then removed by centrifugation. This procedure was repeated three times.

*Experimental.*—1. *Antigenic properties of cultures derived from day-old rat liver:* One of the properties commonly used to describe and characterize a particular cell type is its antigenic structure. It was of interest, therefore, to examine the antigenic relationship between cells that become established in tissue culture, and the tissue of origin of these cells. Accordingly, an inoculum prepared from day-old rat liver was permitted to grow for twelve days. The plates were carefully washed to remove all cells that were not fixed to glass. The attached and stretched cells were freed by treatment with trypsin, harvested, and replated in culture medium in the presence of various rabbit sera. Each plate was inoculated with $10^4$ cells, and the rabbit sera added at a level of 3 per cent (v/v). After further incubation for $2^1/_2$ days, the plates were washed, the remaining cells fixed with 10 per cent neutral formalin and stained with Giemsa, and the attached and stretched cells counted. The rabbit sera employed were (1) pre-immunization serum designated as normal serum, (2) antiserum prepared against purified single cell suspensions of adult rat liver, (3) antiserum prepared against tissue cultures obtained from day-old rat kidney. Separate portions of the three sera were absorbed with either adult rat liver or minced preparations of hepatectomized day-old rats. The results of the experiment are recorded in Table 1. From the data it is apparent that both antisera, when unabsorbed, effectively destroy the ability of tissue culture cells from day-old rat liver to attach and stretch. Of importance, however, was the finding that liver culture cells were susceptible to anti-kidney-culture antiserum which had been absorbed with rat liver, while, at the same time, resistant to the action of anti-liver antiserum absorbed with hepatectomized day-old rat. These results implied that tissue culture populations derived from day-old rat liver dif-

TABLE 1

VIABILITY OF LIVER CULTURE CELLS IN THE PRESENCE OF RABBIT SERUM

| Serum | Unabsorbed | Absorbed with Adult Liver | Absorbed with Hepatectomized Day-Old Rat |
|---|---|---|---|
| | Number of attached and stretched cells per plate | | |
| Normal | 1005 | 2020 | 1750 |
| Anti-adult liver | 22 | 1260 | 1425 |
| Anti-day-old-rat-kidney culture | 9 | 55 | 1300 |

Liver culture cells were harvested by trypsinization and plated in the presence of various rabbit sera. The inoculum was $10^4$ cells per plate and the antiserum level was 3 per cent (v/v). After 60 hr incubation, the plates were washed, fixed with formalin, stained with Giemsa, and the number of attached and stretched cells counted.

fered from liver parenchymal cells in that they lacked certain liver antigens. Moreover, these same populations possessed antigens related to kidney culture antigens, which are absent in liver parenchyma. Of additional interest was the fact that absorption of all antisera with hepatectomized day-old rat eliminated toxicity. This implied that the antigens responsible for antiserum sensitivity of the liver cultures must, therefore, be present in tissues other than liver.

2. *Demonstration of anti-liver antibodies by complement (C′) fixation:* Central to the inference that culture cells differ markedly in antigenic structure from the original parenchymal cells is the assumption that anti-liver antiserum, absorbed with hepatectomized young rat does contain antibodies to the original parenchymal cells in spite of being ineffective against culture cells. This was confirmed by the use of complement fixation. The absorbed antiserum, in dilutions ranging from $^1/_{25}$ to $^1/_{800}$, was set up in block titration with approximately 5 C′ $H_{50}$ units of guinea pig complement.[12] Day-old rat liver cell suspensions were added in tenfold increments from $10^3$ to $10^6$ cells. The final volume in each reaction mixture was 1.3 ml. Veronal buffer with added $Ca^{++}$ and $Mg^{++}$ was used as diluent, and

TABLE 2*a*

COMPLEMENT FIXATION WITH DAY-OLD RAT LIVER AS ANTIGEN AND ANTI-ADULT RAT LIVER ANTISERUM ABSORBED WITH HEPATECTOMIZED DAY-OLD RAT

| As/G | $2 \times 10^6$ | $2 \times 10^5$ | $2 \times 10^4$ | Veronal buffer |
|---|---|---|---|---|
| 1/25 | 0.165 | 0.053 | 0.240 | 0.688 |
| 1/50 | 0.181 | 0.046 | 0.385 | 0.692 |
| 1/100 | 0.695 | 0.059 | 0.470 | 0.702 |
| 1/200 | 0.820 | 0.525 | 0.760 | 0.822 |
| 1/400 | 0.850 | 0.728 | 0.650 | 0.740 |
| Veronal buffer | 0.807 | 0.663 | 0.685 | 0.760 |

TABLE 2*b*

COMPLEMENT FIXATION WITH DAY-OLD RAT LIVER AS ANTIGEN AND ANTI-DAY-OLD-RAT-KIDNEY-CULTURE ANTISERUM ABSORBED WITH ADULT RAT LIVER

| As/G | $2 \times 10^6$ | $2 \times 10^5$ | $2 \times 10^4$ | Veronal buffer |
|---|---|---|---|---|
| 1/25 | 0.505 | 0.695 | 0.695 | 0.732 |
| 1/50 | 0.728 | 0.695 | 0.712 | 0.712 |
| 1/100 | 0.810 | 0.668 | 0.728 | 0.700 |
| 1/200 | 0.932 | 0.720 | 0.735 | 0.710 |
| 1/400 | 0.890 | 0.700 | 0.686 | 0.745 |
| Veronal buffer | 0.686 | 0.712 | 0.688 | 0.688 |

Antisera against adult rat liver and antiserum against cultures of day-old rat kidney were incubated wity liver cells from day-old rats, at 37°C for one hr with frequent agitation. $10^8$ sensitized sheep red cells were added and the incubation continued for another hr. Sedimentable material was removed by centrifugation and the optical density of the supernatant measured at 541 mμ.

appropriate controls included to test for anticomplementary activity at each level of antiserum and antigen. After incubation at 37°C for one hr, $10^8$ sensitized sheep red blood cells were added, and complement fixation determined by the extent of lysis of the red cells. The results are presented in Table 2, where the amount of complement fixed is in inverse relation to the optical density of the lysed erythrocytes. It is seen that anti-adult rat liver antiserum absorbed with hepatectomized day-old rat gave a typical block titration pattern with day-old rat liver. On the other hand, anti-kidney culture antiserum, absorbed with adult liver, gave perceptible complement fixation with day-old rat liver only at the highest levels of antibody and antigen that could be used without being anticomplementary.

3. *Pretreatment of inocula with antisera:* It must be emphasized that the antigenic differences between populations of parenchymal cells and tissue culture cells were, of necessity, observed only after the period of growth required to obtain a culture population. In general, this took about two weeks, and it was of interest to inquire into the mode of origin of these antigenic differences. There were at least two possibilities. First, the parenchymal liver cells of the young rat underwent antigenic modification during the *in vitro* culture period. Alternatively, the antigens present after two weeks in culture were present initially, in the inoculum,

TABLE 3

PRETREATMENT OF DAY-OLD RAT LIVER INOCULA WITH ANTISERA

| Serum | Unabsorbed | Absorbed with adult liver | Absorbed with hepatectomized day-old rat |
|---|---|---|---|
| | Number of attached and stretched cells per plate | | |
| Normal | 1850 | 3000 | 1740 |
| Antiadult liver | 1200 | 1000 | 1000 |
| Anti-day-old-rat-kidney culture | 3 | 38 | 1800 |

Fresh inocula prepared from day-old rat liver were incubated at 37°C for 30 min in the neutral synthetic medium supplemented with antisera at 5 per cent (v/v) and 3 C′ H50 units of guinea pig complement per ml. The inocula were centrifuged and washed to remove unreacted antibody and complement. An amount of tissue equivalent to $10^6$ cells were inoculated into each plate containing the standard medium. After a week of growth the plates were washed, fixed and stained, and the attached and stretched cells counted.

on cells different from the parenchymal cells and capable of growth in culture. To examine these proposals, it was planned to pretreat fresh inocula of day-old rat liver with the identical set of rabbit antisera employed in the previous experiment, then plate in the absence of antiserum. It could be predicted that if it were the parenchymal cells that were modified, then growth should be blocked by anti-liver antiserum. If, on the other hand, the antigens were there initially, then anti-culture antiserum absorbed with liver should inhibit growth. In the experiment, inocula of day-old rat liver were incubated at 37°C for 30 min in the neutral standard medium supplemented with antisera at 5 per cent (v/v), and 3 C′ $H_{50}$ units of guinea pig complement per ml. The inocula were centrifuged and washed to remove unreacted antisera and complement, and plated in the absence of antisera in the standard medium. Each plate received an inoculum equivalent to $10^6$ cells. A week later the plates were fixed and stained, and the number of attached and stretched cells per plate counted. The results presented in Table 3, demonstrate that the cells destined to give rise to the final tissue culture population had the same spectrum of antiserum sensitivity as did the final population.

4. *Ornithine transcarbamylase content of tissue culture derived from day-old rat liver:* The results obtained in the serological studies were entirely consistent with

the possibility that liver tissue culture populations originated from a cell type other than the liver parenchyma. To examine this possibility further, additional properties associated with liver parenchymal cells were investigated. It has been shown that in the rat, the enzyme, ornithine transcarbamylase, is restricted to the liver.[9] It is found in but few other tissues and there in low specific activities.** A study was made, therefore, of the ornithine transcarbamylase content of day-old rat liver and derived tissue cultures.

In this experiment, two-week old cultures, grown in the presence of ornithine (0.1 mg/ml) were harvested by the following procedure: the Petri dishes were repeatedly washed with saline to remove as much of the loose cells and pieces of tissue (nongrowing portion of inoculum) as possible. The cells were then covered with the final suspending fluid (0.9% KCl, 0.001 *M* glutathione) and scraped with a rubber policeman to remove the cells adhering to glass. This suspension was then

TABLE 4

ORNITHINE TRANSCARBAMYLASE ACTIVITY OF LIVER-DERIVED TISSUE CULTURES

| Material | Ornithine-transcarbamylase activity in micromoles of citrulline per mg protein per twenty min |
|---|---|
| Two-week-old liver cultures | 0.5 |
| Fresh day-old rat liver preparation (inoculum) | 10.0 |
| Adult rat liver (whole) | 10.0 |
| Purified single cell suspension of adult rat liver | 20.0 |

Two-week-old liver cultures were assayed for ornithine transcarbamylase activity by the method of M. E. Jones. The cells were harvested, by first washing with saline to remove loose cells and tissue. The final suspending fluid was added and the glass-attached cells removed by scraping with a rubber policeman.

ground in a Potter-Elvejhem homogenizer and the homogenate assayed for ornithine transcarbamylase. The results are presented in Table 4. The activities of whole adult liver, of a purified single cell suspension of adult liver parenchyma, and of fresh day-old rat liver preparation (the inoculum) are presented for comparison. The experiment showed that the ornithine transcarbamylase activity of liver cultures was low, with a value only 5 per cent that of the inoculum used to initiate the culture. It was found that this residual activity could be attributed to the persistence of nongrowing portions of the inoculum in spite of the strenuous efforts to wash them away. As the next experiment demonstrates, ornithine transcarbamylase does persist at high levels in nongrowing cells *in vitro*, particularly in the presence of ornithine.

5. *The persistence of ornithine transcarbamylase in nongrowing cells in vitro:* Single cell suspensions of adult liver were inoculated into standard culture medium to which ornithine at 0.1 mg/ml was added. The cells were then periodically assayed for ornithine transcarbamylase. (Under none of a wide variety of cultural conditions has an increase of cell number ever been observed with adult liver cell preparations.) Preparations of day-old rat liver were incubated in the same manner with replicate cultures from which the ornithine was omitted. Although growth was obtained with the young rat preparation, only the nongrowing, loose, and unattached cells were assayed. The results are presented in Figure 1 where it is seen that ornithine transcarbamylase persisted in nongrowing cells *in vitro* for relatively long periods. In the presence of ornithine, ornithine transcarbamylase activity in both adult and young rat liver preparations was still 50 per cent that of the inoculum at the end of two weeks. In the absence of ornithine, enzymatic

activity dropped more sharply but was still about 20 per cent that of the inoculum.

6. *The serum albumin content of liver culture cells:* Since the mammalian liver is known to be the site of serum albumin biosynthesis, it was of interest to examine liver-derived cultures for serum albumin content. Ten-day old cultures of day-old rat liver were harvested by the method used in the ornithine transcarbamylase assays. The attached and stretched cells, and the loose, nongrowing portions of the inoculum were assayed separately for serum albumin, by complement fixation with rabbit anti-rat-serum-albumin antiserum prepared by Dr. Lawrence Levine. For the assay the cells were disrupted by sonic oscillation. Freshly prepared liver from perfused day-old rats was assayed in the same manner after exhaustive washing with saline to a constant serum albumin value. The values obtained for fresh liver, nongrowing cells, and growing cells in micrograms of serum albumin per milligram of protein were 2.3, 2.7, and 0.4, respectively. The results parallel those obtained with ornithine transcarbamylase, in that the differentiated property persists in the nongrowing cells and decreases in the growing cells. Again, the residual serum albumin in the growing cells can be attributed to the persistence of portions of the inoculum.

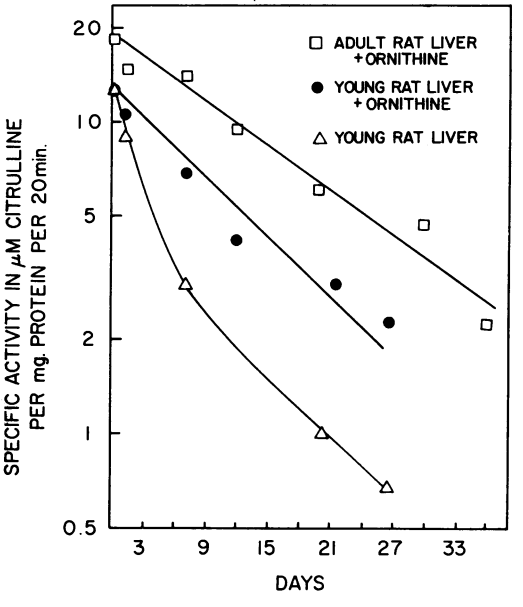


FIG. 1.—Single cell suspensions of adult rat liver parenchyma were incubated in the standard medium supplemented with 0.1 milligrams of ornithine per ml. The standard preparation of day-old rat liver was incubated in the same manner, with replicate plates from which ornithine had been omitted. From day to day the loose, unattached cells were assayed for ornithine transcarbamylase.

*Discussion.*—The major experimental findings presented in these studies are that: (1) tissue culture populations derived from day-old rat liver differ markedly in antigenic structure from day-old liver, (2) freshly prepared liver inocula are prevented from initiating culture growth by pretreatment with kidney tissue culture antiserum which has been absorbed with liver. Moreover the growth potential of liver inocula is unaffected by anti-liver antiserum containing demonstrable antibodies against liver. In addition, short term experiments reveal that liver cultures possess sharply reduced ornithine transcarbamylase activity and serum albumin content. The residual enzyme activity and albumin content can be accounted for by the microscopically demonstrable persistence of portions of the inoculum in these experiments.

We conclude from these facts that the bulk of the tissue culture population derived from day-old rat liver arises from a cell type other than the parenchymal cell. That this cell constitutes a small minority of the total cell population is supported by the finding in our laboratory, to be reported in detail elsewhere, that single cell

suspensions of day-old liver have a plating efficiency of approximately $10^{-4}$. In a forthcoming publication, evidence will be presented that this figure is a true measure of the fraction of cells of the day-old rat liver which are capable of growth in the conventional tissue culture situation.

The general lack of differentiated properties in established tissue culture strains suggests that a common basis exists for this phenomenon. It is not unreasonable that the usual methods of obtaining cultures from freshly excised tissue should selectively favor a minority, nonparenchymal cell type. The usual procedure is to start with large sections of tissue. The sections are planted in culture media which have been designed to support the growth of strains isolated in this particular manner. Since populations arise in these procedures by a mixed process of migration from the slice, and cell division, it is probable that the cell types favored should be those that are normally motile, not tightly bound in tissue structure, and which can accommodate themselves to the artificial conditions of *in vitro* growth.

Our experiments do not exclude the possibility that liver parenchymal cells, if successfully cultured, would gradually lose their differentiated properties. The recent work of Schindler, Day, and Fischer,[13] in which cloned cultures of cancerous mast cells were shown to retain their full capacity to synthesize histamine and serotonin, present at least one example of differentiated cells maintaining differentiated properties in culture.

A wealth of literature[14] exists purporting to show the growth of differentiated cells while at the same time established strains of such cells are in short supply. This disparity can probably be explained by the limitations involved in the experimental methods employed. In many cases short term experiments are used in which the nongrowing portions of the inoculum are assayed along with the growing cells of the culture for differentiated properties, while in others properties are studied which are not strictly specific to the tissue of origin. The first danger is most prominent in embryological experiments. Tissues, after a brief *in vitro* sojourn are reinjected into animals and give rise to differentiated structures corresponding to the tissue which was cultured. The conclusion that the structure was derived from differentiated cells which had multiplied in culture is suspect, since it is difficult in such studies to obtain a measure of the number of cells required to initiate the structure, and a small contamination of the cultures with nondividing but surviving portions of the inoculum could account for the results. Our studies on the persistence of ornithine transcarbamylase in nongrowing cells *in vitro* show how easily this possibility may be realized. The presence of arginase, rhodanase, and glycogen has been offered as evidence by Earle and his associates[15] that certain of their strains are derived from liver parenchyma. Although these properties are most prominent in liver, we feel that the relative degree of specificity afforded by these criteria is insufficient for a final conclusion because these properties singly and in combination have been found in tissue cultures of nonliver origin.[15-17]

The conclusion that liver cultures lack liver properties because they are descended from non-liver cells suggests that some of the difficulties in tissue culture isolation procedures can be overcome by techniques designed to suppress the growth of the contaminating cell type. Antiserum specific for this hypothetical cell type can be used in enrichment culture procedures and, in the case of liver, advantage can be taken of the fact that the liver is capable of synthesizing those nonessential amino

acids such as arginine which are found to be universally required for culture growth. These procedures are now being employed in our laboratory, but to date no survivors to anti-tissue culture serum have been found, and no cells have been isolated from liver which are arginine-independent. We feel that this may reflect a far more serious obstacle in the culture of differentiated cells. In the case of liver,[18] it is known that simple removal of liver from the body results in an immediate and drastic hydrolysis of the pyridine nucleotide coenzymes in the excised lobe. It is possible that biochemical lesions of a similar lethal effect may occur in many differentiated cells as a result of excision.

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**Ornithine transcarbamylase activity of various rat tissues, from M. E. Jones *et al.* (unpublished data):

| Tissue | O.T.C. $\mu M$ citrulline/gm. fresh tissue/hr | Tissue | O.T.C. $\mu M$ citrulline/gm. fresh tissue/hr |
|---|---|---|---|
| Liver | 4400 | Spleen | 0 |
| Intestinal mucosa | 100 | Lung | 0 |
| Kidney | 5 | Plasma | 0 |
| Salivary gland | 12 | Plasma cells | 0 |
| Brain | 0 | Skeletal muscle | 0 |
| Testis | 0 | Adrenal | 0 |
| Thymus | 0 | Thyroid | 0 |
| Pancreas | 0 | | |

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# *GENETIC CONTROL OF PHOTOSYNTHESIS IN CHLAMYDOMONAS REINHARDI**

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An increasing knowledge of the physical and chemical events which attend the process of photosynthesis opens the way to an investigation of genetic controls of this fundamental phenomenon and permits us to consider the question of the degree of control over photosynthesis exerted by the nucleus and the chloroplast.

Genetic controls of photosynthesis can be envisaged as preceding along two pathways. First, the controls can be direct. For example, there may be genetic control over the synthesis of enzymes specifically involved in photosynthetic reactions. These controls may be independent of a second pathway in which genetic changes in chloroplast structure so alter the site of photosynthesis as to affect numerous photosynthetic reactions.

There are, at present, forty-two UV-induced mutants of the sexually reproducing unicellular green alga, *Chlamydomonas reinhardi*, which cannot grow in the light unless the minimal medium is supplemented with sodium acetate. Each of the mutants represents a simple genetic change in that it segregates in a one-to-one fashion when crossed to wild-type.

The requirement for a carbon source other than or in addition to carbon dioxide suggests at least three possibilities for genetic blocks in these mutants. First, the acetate mutants may be unable to carry out certain of the light-requiring reactions of photosynthesis and thus possess genetic blocks which lie along the first pathway mentioned above. Second, the acetate mutants may have altered pigments or they may be pigment-deficient, and therefore are mutants which act indirectly on photosynthesis by way of the second pathway. Third, the mutants may possess metabolic blocks at some non-photosynthetic step in their intermediary metabolism.

One mutant strain, *acetate-21* (*ac-21*), appears to be incapable of sufficient carbon dioxide fixation to grow photosynthetically, and thus it falls into the category of a mutant strain which may be blocked in some essential step of photosynthesis.