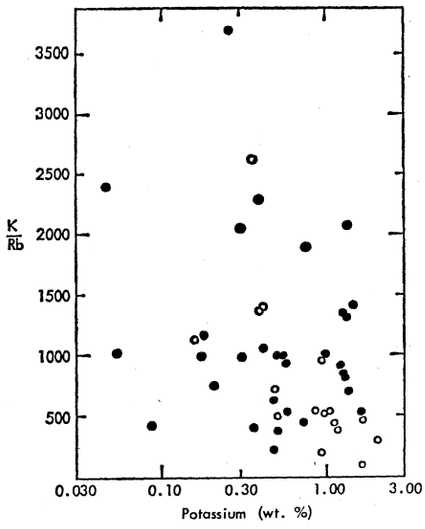

Fig. 1. Potassium : rubidium ratios and potassium contents of amphiboles. Open circles, igneous samples; solid circles, metamorphic samples.

relation between K:Rb ratio and potassium content as might be expected if the potassium content of the amphibole reflected the relative position of the host rock with respect to differentiation history. The K:Rb ratio generally tends to decrease with increasing differentiation (*1–5*). Our samples represent a very heterogeneous population of rock types, however, and any real trends to be expected in Fig. 1 may be obscured for this reason.

We feel that the above-mentioned data may have a particular bearing on the anomalously high K:Rb ratios reported in abyssal tholeiites (*2, 4*). Gast (*2*) and Engel and Engel (*3*) state that the K:Rb ratio of the mantle must be at least as high as that in the tholeiites that are derived from it, and that the upper mantle therefore is chemically more like achondritic (K:Rb $>$ 1000) than chondritic (K:Rb $\sim$ 300) meteorites. The ambiguity of this approach can be easily shown by adopting a mantle model such as Ringwood's (*18*), which has an amphibole peridotite as the stable assemblage in the uppermost mantle. Cogent arguments in favor of amphibole as a phase in the upper mantle have been presented (*19*).

The melting relations of amphibole peridotite are essentially unknown; it is likely however that the hydrous phase will be one of the earliest to melt, perhaps incongruently (*20*). The liquid produced by partial melting of an amphibole peridotite would then contain most of the alkalis originally in the amphibole. In the case of the Saint Paul Rocks peridotite, the K:Rb ratio of this early liquid would not only exceed 1000, but it would also be greater (more "primitive" appearing) than the K:Rb ratio of the material from which it was derived. Clearly, the K:Rb ratios of abyssal tholeiites cannot be used a priori to set lower limits on the K:Rb ratio of the tholeiite-source regions.

So far we have considered only closed-system partitioning between the minerals of a given assemblage; we may also speculate on the large-scale effects of partitioning between assemblages. Ringwood's pyrolite model, for example, proposes an upper amphibole-peridotite layer that transforms at depth to peridotite or garnet peridotite (*18*). Any equilibration of trace elements between these layers would result in an upward transfer of potassium relative to rubidium, resulting in a higher K:Rb ratio in the uppermost mantle than in the deeper regions. Similar effects may exist for other trace elements such as Sr, U, Th, and Pb, all of which figure prominently in discussions of mantle composition. If Rb were relatively impoverished in the upper mantle, the Rb:Sr ratio of the mantle might even increase with depth, invalidating one of the main arguments against a chondritic earth model (*21*). Clearly, compositional models for the mantle, based on trace-element data from derived material such as basalt, must recognize the very real control that mineralogy can have on trace-element chemistry.

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10. Separated minerals donated by H. H. Hess. TQ2 mode: 45 percent hornblende, 28 percent pyroxene (dominantly clinopyroxene), 25 percent plagioclase (An 70), and 2 percent ores.
11. Mode for B65 [from (*12*)]: 45 percent plagioclase (An 30), 37 percent hornblende, 9 percent diopside, 4 percent microcline, 2 percent epidote, 2 percent sphene, and traces of quartz and apatite.
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Phenotypic Variations among Chloroplasts of a Single Cell

Abstract. *Some normal-looking chloroplasts of a* Nitella *cell do not incorporate carbon dioxide into substances insoluble in cold acid. The distribution of such chloroplasts among the rows of normal organelles indicates that the defect is not due to a genetic mutation but probably represents a physiological stage in the development of the organelles.*

The presence within a single cell of diverse types of mitochondria or plastids is considered good evidence for the existence of an independent genetic system in these organelles (*1*). Hagemann (*2*) described mixed cells, containing green and white plastids, in border regions between green and white areas of leaves of variegated plants. Avers *et al.* (*3*) demonstrated differences within the population of mitochondria of a single yeast cell by the diamine cytochemical test for cytochrome oxidase (*4*). They found that only some of the mitochondria gave a positive reaction for activity of cytochrome oxidase. Ogawa and Barrnett (*5*) showed differences among the mitochondria of cells from rat heart by use of tetrazolium salts. The accumulation of formazan crystals over

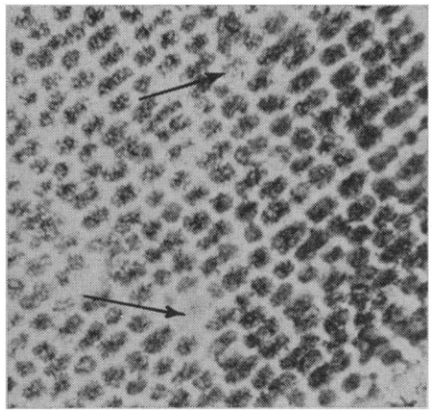

Fig. 1. Fixed rows of chloroplasts from a *Nitella* cell after 30-minute exposure to $C^{14}O_2$ in light and 3 days of autoradiography. Silver grains over the plastids indicates the accumulation of C^{14}. Arrows indicate chloroplasts which did not fix carbon (approximately × 550).

some mitochondria and not over others indicated differences in the respiratory activity of these organelles.

The existence of such biochemical differences between organelles is, however, not a sufficient indicator for genetic differences between the organelles. One must show whether the differences are perpetuated in subsequent divisions of the organelles or whether they represent physiological stages in the development of the organelles. My findings indicate that biochemical differences exist among plastids of a single cell of *Nitella*, but that these differences are probably phenotypic and not genotypic.

I chose to study *Nitella*. In a cinematographic study of the developing cells of *Nitella*, Green (*6*) demonstrated the regular arrangement and divisions of the chloroplasts of these cells. The pattern of chloroplast divisions results in regular rows of organelles. These rows, consisting of hundreds of chloroplasts, therefore represent "geneologically" related organelles. If a genetic mutation of a plastid occurs, it will result in a row of modified plastids. The length of this row should indicate when in the life of the cell the mutation occurred.

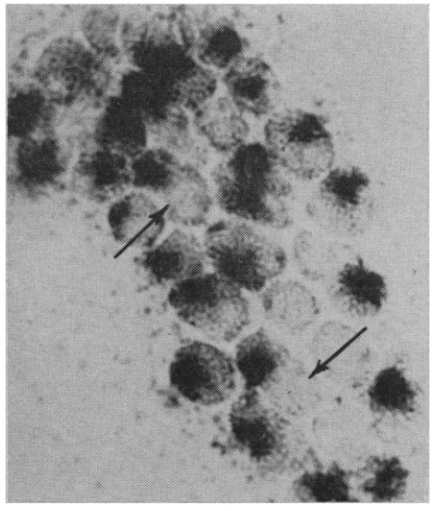

Fig. 2. Short segments of rows of chloroplasts, after 6 days of autoradiography, in which several dividing chloroplasts are seen (arrows). Note the concentration of radioactivity in one of the poles only.

Nitella is suitable for such studies because the plastids are attached to a layer of cytoplasm that does not stream. The same plastids can therefore be observed closely for the duration of several plastid generations.

In an attempt to discover genetically different chloroplasts in *Nitella* cells, I performed experiments to determine the photosynthetic capabilities of the chloroplasts. Live, growing cells were immersed in a solution of 0.005*M* phosphate buffer, *p*H 7, containing soil-extract and to which C^{14} as Na_2CO_3 (10 μc/ml) was added. (The specific activity of the carbonate was 20 mc/m*M*.) Experimental cells were exposed to light (about 5500 lumens/m^2, from several white fluorescent lamps) for 30 minutes, while control cells were kept in an identical solution in the dark. To terminate the experiment, I removed the cells from the radioactive solution, washed them in water, and fixed them in a 2 percent glutaraldehyde solution for 3 hours. The cells were washed again and cut into segments 1 to 2 mm long. These segments were gently squashed onto glass slides. Flat layers of organized rows of plastids could thus be obtained and affixed to the glass surface. The slides were dried, washed in 1 percent acetic acid, washed again in water, and coated with a photographic emulsion (Ilford K 5) for autoradiography.

The slides were developed after several days and examined for the distribution of silver grains. Figure 1 is of a layer of plastids from a cell kept in light. The concentration of silver grains over most of the chloroplasts is clearly seen. At the arrows are chloroplasts which apparently did not fix noticeable quantities of carbon into substances insoluble in cold acid. Cells kept in the dark did not contain detectable amounts of C^{14}.

As judged by light microscopy, all the chloroplasts appeared similar.

It is apparent, however, that not all the plastids are identical in their biochemical activities. Preparations stained with iodine had high concentrations of radioactivity associated with the presence of starch grains in the plastids.

The deficient chloroplasts appeared to be randomly distributed; at no time were rows or segments of rows seen in which all the chloroplasts were defective. In some cases (Fig. 2) dividing chloroplasts were seen in which carbon was fixed by one of the daughter plastids and not by the other.

From these results I conclude that the observed differences between the plastids are not due to genetic differences between them but probably to unequal distribution of the enzymatic apparatus for starch synthesis between daughter plastids. The plastid which received the "old" starch grain and its associated enzymes is capable of efficient fixation of carbon. The other plastid is to be regarded as physiologically "younger"; it will have to develop its apparatus for starch synthesis. It is also possible that the nonsynthesizing plastids are defective and will not develop further.

In many algal species, synthesis of starch or similar carbohydrate reserves is associated with a specialized region of the chloroplasts, the pyrenoids. Such specialized regions have not been described in plastids of higher plants, and the chloroplasts of *Nitella* are also devoid of pyrenoids (*7*). However, an intimate association of starch-synthesizing enzymes with starch grains was established for higher plants (*8*).

Despite the absence of pyrenoids in the chloroplasts of *Nitella*, it appears likely from these results that specific areas of the chloroplast are specialized for starch synthesis.

Very little is known as yet about the organization of enzymes within chloroplasts. However, the carbon-fixing system of the chloroplast is thought to be localized in the stroma —that is, between the chlorophyll-bearing lamella. It appears that these enzymes of the stroma are also spatially organized in the organelle.

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L-Asparaginase: Toxicity to Normal and Leukemic Human Lymphocytes

Abstract. *Quantitative in vitro tests showed that purified preparations of* L-*asparaginase from* Escherichia coli *were more toxic to blood lymphocytes from 12 of 15 patients with chronic lymphocytic leukemia than to lymphocytes from 25 persons with normal hemograms. Incubation for 7 days with 10 units per milliliter killed, on the average, 77 percent of leukemic and 34 percent of normal lymphocytes. The reagent produced appreciable toxicity to leukemic lymphocytes after 2 days of incubation.*

Guinea pig serum has been shown to cause regression of transplantable mouse lymphoma 6C3HED (*1*) and of transplants from spontaneous mouse lymphomas (*2*). Broome (*3*), Old *et al.* (*4*), and Holmquist (*5*) produced evidence that L-asparaginase may be the antilymphoma factor in guinea pig serum. Asparaginase isolated in purified form from guinea pig serum produced regression of 6C3HED (*6*). Mashburn and Wriston (*7*) and Dolowy *et al.* (*8*) demonstrated that L-asparaginase obtained from *Escherichia coli* also produced regression of 6C3HED lymphomas. By quantitative in vitro methods, Schrek (*9*) found that guinea pig serum had a greater cytocidal effect on lymphocytes from patients with chronic lymphocytic leukemia than on lymphocytes from healthy individuals. Our study measured the cytocidal effect of L-asparaginase from *E. coli* on normal and leukemic lymphocytes.

Purified suspensions of blood lymphocytes were prepared by methods described previously (*10*). The medium consisted of equal parts of normal human serum and Fischer's medium No. 147G; both serum and medium contained 10 μg of asparagine per milliliter (*11*). Suspensions with and without L-asparaginase were incubated in test tubes for 2 to 7 days. To obtain counts of viable lymphocytes, 0.2 ml of suspension was placed in a special slide chamber that consisted of two large cover slips (45 by 50 mm) separated by a metal disk (40 by 40 mm by 1 mm in thickness) with a central hole 25 mm in diameter. Cells were examined with an inverted phase contrast microscope and the number of viable lymphocytes in an area 10 by 0.04 mm was counted. Viable lymphocytes were counted in treated and untreated suspensions before and after incubation. Cytologic criteria used for determining viability were developed by studying time-lapse cinemicrographic films (*10, 12*). In brief, a lymphocyte was considered viable if it had a morphologically intact nucleus with a thin nuclear wall and chromatin masses or a nucleolus. Other criteria of viability were elongation or irregularity in the shape of the cell and the presence of a pseudopod or of cytoplasmic projections. A typical dead lymphocyte, in this study, had no nucleus but was round with a thin cellular wall, a few cytoplasmic granules, and an ill-defined clear space. A few dead cells had homogeneously gray pyknotic nuclei without any discernible structure. The percentage of lymphocytes that survived incubation was based on the number of viable lymphocytes in the original suspension before incubation; the percentage killed by the reagent (or percent cytocidal effect) was based on the ratio of the numbers of viable lymphocytes in treated and untreated suspensions after a given period of incubation.

L-Asparaginase from *E. coli* was assayed according to a method described previously (*13*); protein concentration was determined by the method of Lowry *et al.* (*14*). Specific activity (micromoles of NH_3 produced from L-asparagine per hour per milligram of protein) of the preparations used ranged from 295 to 398.

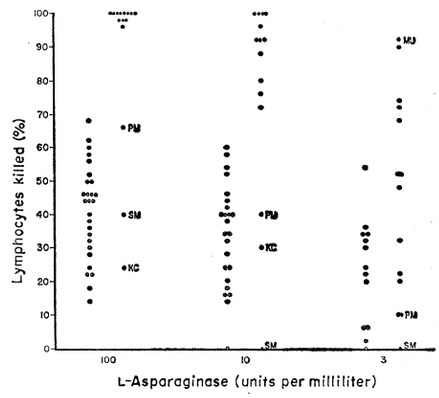

Fig. 1. The percentage of lymphocytes killed in vitro by incubation for 7 days with 3, 10, and 100 units of L-asparaginase per milliliter. This percentage was based on the number of lymphocytes that survived in the control suspensions incubated for 7 days without L-asparaginase. Lymphocytes were derived from 25 persons with normal hemograms (open circles) and from 15 patients with chronic lymphocytic leukemia (closed circles).

Suspensions of blood lymphocytes from 25 persons with normal hemograms and from 15 patients with chronic lymphocytic leukemia were incubated for 2, 5, and 7 days with 1, 3, 10, and 100 units of L-asparaginase per milliliter (see Table 1). At all dosages and time intervals tested, the leukemic lymphocytes were more sensitive than normal lymphocytes. At 10 units per milliliter, the reagent produced only a minimal effect on normal lymphocytes after 5 days of incubation but killed many of the leukemic lymphocytes in 2 days, and most of them in 5 days. This dose (10 units of asparaginase per milliliter) is of particular interest since Dolowy *et al.* (*15*) showed that while

Table 1. Effect of L-asparaginase on blood lymphocytes from persons with normal hemograms and from patients with chronic lymphocytic leukemia. Only the first test on a patient is used in this table.

Incubation time (days)	No. of persons tested	Lymphocytes killed (%) by incubation with L-asparaginase (units/ml)*				Viable lymphocytes (%) in untreated suspension†
		100	10	3	1	
		Normal lymphocytes				
2	7	10.4				87.0
5	14	19.3	13.5			59.4
7	25	41.0	33.9	22.0	8.1	50.7
		Leukemic lymphocytes				
2	12	68.5	18.7			71.1
5	13	85.0	66.5			64.1
7	15	88.0	76.9	45.7	11.5	61.7

* Percentage of lymphocytes killed equals 100(1 — No. viable lymphocytes in treated, incubated suspension/No. viable lymphocytes in untreated, incubated suspension). † Percentage of lymphocytes surviving in untreated, incubated suspension is based on the number of lymphocytes in the original suspension before incubation.