Table. 1. Genetic markers contributed to P221 by P22.

P22 stock*	Markers found in P221 isolated from stock
P22$c_2h_{21}^+$	P221$c_2h_{21}^+$
P22$c_3h_{21}^+$	P221$c_3h_{21}^+$
P22c_1h_{21}	P221c_1h_{21}
P22$m_3c_3h_{21}^+$	P221$c_3h_{21}^+$
P22$m_3c + h_{21}$	P221$c + h_{21}$
P22g	P221g

* Stocks of the various P22 mutants listed in the left hand column were prepared and plated on St/22 for the isolation of P221 strains.

found by the following procedure. The lysate of mixed infection of St/22 with P22*h* and P221 was filtered through a 0.45-μ Millipore filter to remove bacteria and debris. The filtrate was then treated for 30 minutes with rabbit anti-P221 serum diluted to give $k_e = 5$ min^{-1}, the velocity constant of neutralization. The treated lysate was then assayed on St/22 with top agar containing anti-P221 serum diluted to give $k_e = 0.8$ min^{-1} to inhibit the development of the P221 phage which was masked by the P22*h* capsid. After overnight incubation at 37°C, clear, pinpoint plaques were found. The size of surviving plaques seemed to be suppressed by anti-P221 serum in top agar. The ratio of these survivors to the original titer of the bacteriophage was about 10^{-6}. Among the 2498 clones of these survivors, isolated and tested with antisera, 2496 clones were P221. Two clones were antigenically P22*h* and showed no cross reaction with the anti-P221 serum. Also, they were morphologically indistinguishable from P22*h*. They were named P22-221Hy. P22-221Hy forms clear, pinpoint plaques on St/22 but no plaques on St. However, St adsorbs P22-221Hy rapidly. From the foregoing it is concluded that St is immune to P22-221Hy. In addition, St/22(P221) is immune to P22-221Hy.

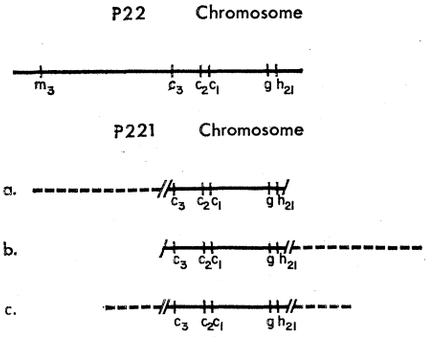

Fig. 1. Three possible structures of P221 chromosome. Solid line indicates region homologous to P22 chromosome. Dashed line indicates region nonhomologous to P22 chromosome. The symbols (/ and //) indicate uncertain location of end of the homologous region. The P22 chromosome was mapped by Levine and Curtiss (*3*).

A number of stocks was prepared from confluent-lysis plates originating from many single clones of P22-221Hy. Every stock of P22-221Hy contained a few percent of both P221 and P22*S*. Unlike P22*h*, P22*S* gives clear plaques on both St and St/22 strains, but it is morphologically and serologically indistinguishable from P22 and P22*h*. P22-221Hy is a stable hybrid rather than a heterozygote, because it does not segregate to P221 and P22*h* by passage through host cells. To further verify this phenomenon, the single-plaque clone technique (*5*) was applied. P221 and P22*S* were found in every plaque clone of P22-221Hy in a frequency of 10^{-4} and 10^{-5}, respectively. There is no explanation for the high frequency of P221 and P22*S* in stocks prepared from confluent-lysis plates.

To eliminate the possibility of P221 contamination, anti-P221 serum was used both in dilution test tubes and in top agar for single cloning technique of P22-221Hy. A number of isolates from a few passages with this treatment still gave the same results. A total of 120 clones of P22-221Hy were tested. All of them bred true, producing P221 and P22*S* in a frequency of 10^{-4} and 10^{-5}, respectively. P221 and P22*S* derived from P22-221Hy carried the same *c* marker and h_{21} color indicator marker. From these results, it may be concluded that P22-221Hy mutates to P221 and P22*S* in a high frequency.

It seems probable that P22-221Hy phage carries a genome which is capable of synthesizing two different phages (or two different phage capsids) but which is masked by only P22*h* capsid. The genetic information for making P221 capsid may be suppressed by that for making P22*h* capsid. To my knowledge, a similar hybrid has not been previously demonstrated for any other phage system.

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Fluoroacetate Inhibition of Amino Acids during Photosynthesis of Chlamydomonas reinhardti

Abstract. *Fluoroacetate inhibited respiration in* Chlamydomonas reinhardti *and greatly decreased the photosynthetic incorporation of* $C^{14}O_2$ *into glutamate and aspartate. This suggests that the Krebs cycle is important in the light-mediated synthesis of glutamate and aspartate in this alga.*

Although the photosynthetic incorporation of $C^{14}O_2$ into amino acids of *Scenedesmus* and *Chlorella* has been previously studied (*1*, *2*), many basic aspects of this problem have not been clarified for algae. Kinetic experiments on $C^{14}O_2$ fixation in *Chlorella pyrenoidosa* (*2*) during steady-state photosynthesis indicate that the formation of glutamate by way of glutamic dehydrogenase may not be the primary route of ammonium incorporation into the amino acids. Two lines of evidence were presented which favor this conclusion: (i) the rate of alanine synthesis was greater than that which could be accounted for by the transamination of pyruvate with glutamate, and (ii) the rate of synthesis of citrate was too low to account for the observed rate of synthesis of glutamate. It was, therefore, suggested that alanine might be synthesized directly from the reductive amination of a 3-C compound and that glutamate might be synthesized by a pathway not involving the Krebs cycle or α-ketoglutarate.

The enzyme alanine dehydrogenase, in the unicellular green alga *Chlamydomonas reinhardti* (*3*), catalyzes the formation of alanine from pyruvate and ammonium but is inactive with phosphoenolpyruvate as the substrate. This enzyme could, therefore, account for much of the synthesis of alanine in *Chlamydomonas*. We also reported the presence of glutamic dehydrogenase (*3*).

The purpose of the present investigation was to assess the importance of glutamic dehydrogenase in the photo-

Table 1. Effect of fluoroacetate on $C^{14}O_2$ fixation (count/min) in *Chlamydomonas reinhardti*.

Compound	Light		Dark	
	Control	Fluoroacetate	Control	Fluoroacetate
Aspartate	106,200	31,010	9,327	4,333
Glutamate	194,346	11,738	7,685	2,894
Serine	74,154	71,987	888	768
Glycine	10,337	14,723	0	0
Alanine	25,833	22,022	1,590	1,065
Glutamine	11,732	10,297	0	0
Asparagine	7,368	0	0	0
Histidine	13,180	12,447	0	0
Cysteine	3,858	4,808	0	0
Proline	9,700	24,095	0	0
Valine	10,474	6,973	0	0
Leucine	7,953	4,370	0	0
Peptide	15,383	3,516	0	0
Citrate	912	258,245	0	48,012
Malate	49,074	25,173	1,623	3,738
α-Ketoglutarate	1,737	0	0	0
Succinate	2,922	0	0	0
Fumarate	2,307	0	0	0
Glycerate	2,541	3,936	0	2,070
Glycolate	6,801	8,410	2,012	375
PGA	129,122	135,282	4,274	14,360
Total	685,934	649,032	27,399	77,603

synthetic incorporation of $C^{14}O_2$ into glutamate. It was also considered desirable to determine the importance of the sequence of reactions generally associated with the Krebs cycle in the light-mediated synthesis of the primary amino acids in *Chlamydomonas*.

Synchronous cultures of *C. reinhardti* (*4*) were grown (*5*) and cells were harvested 12 hours after division—a time at which $C^{14}O_2$-fixation was at a maximum. All cells were thus in the light for at least 12 hours prior to experimentation. For each experiment 0.25 ml of packed cells were resuspended in 25 ml of fresh culture medium. One hour prior to incubation with $NaHC^{14}O_3$, fluoroacetate ($1 \times 10^{-3} M$) was added to the cell suspension. This treatment inhibited the rate of respiration of the cells by 75 percent. The fluoroacetate-treated cells and untreated controls were then incubated in the light (3000 lux) or in the dark with 200 μc of $NaHC^{14}O_3$ for 10 minutes at 25°C. The cells were killed and extracted by the addition of 75 ml of boiling 80 percent ethanol.

The alcohol-soluble compounds were separated with Dowex 50(H) and Dowex 2 (formate) into amino acid and organic acid fractions, respectively. The compounds in each of these fractions were further fractionated by two-dimensional paper chromatography (*6*). Radioautographs were obtained by overlaying the developed chromatograms with Kodak no-screen x-ray film for approximately 1 week. The radioactive spots were cut from the chromatograms and the radioactivity estimated in a scintillation counter (*7*).

The results of these experiments (Table 1) may be summarized as follows: (i) fluoroacetate caused a 270-fold accumulation of labeled citrate in the light; (ii) the synthesis of all detectable radioactive Krebs-cycle intermediates other than citrate was markedly inhibited by fluoroacetate; (iii) the synthesis of labeled glutamate was inhibited by 94 percent; (iv) the synthesis of labeled aspartate was inhibited by 70 percent with fluoroacetate; (v) the incorporation of C^{14} into alanine was not affected by fluoroacetate; (vi) the total amount of C^{14} incorporated into the soluble compounds was the same with or without the inhibitor in the light, but three times greater with the inhibitor in the dark.

The fact that the fluoroacetate had no effect on the total amount of radioactivity incorporated by the cell indicates that this inhibitor has no effect upon the initial steps of CO_2 reduction. The accumulation of citrate, the decrease in respiration, and the disappearance of radioactive Krebs-cycle intermediates are all taken as evidence that fluoroacetate is metabolized to fluorocitrate which inhibits the enzyme aconitase. In the dark, the presence of fluoroacetate results in increased labeling of the soluble compounds compared to those in the dark control. This, in all probability, is due to the inhibition of $C^{14}O_2$ evolution by way of the Krebs cycle. In the light, this inhibition would be obscured because of the greater rate of CO_2 fixation.

The fluoroacetate inhibition of the photosynthetic C^{14} incorporation into glutamate is indicative that most of this amino acid arises from a pathway related to the Krebs cycle.

The synthesis of aspartate and malate are also inhibited by fluoroacetate but not to the same extent as glutamate. This can be attributed to the carboxylation of phosphoenolpyruvate, resulting in the formation of oxalacetate which may be transaminated to yield aspartate or reduced to malate. The enzymatic reactions required for this sequence in *C. reinhardti* have been demonstrated in vitro (*8*). Nevertheless, the inhibition observed indicates that the synthesis of these two compounds is also contingent upon the operation of the Krebs cycle.

The fact that the synthesis of alanine is not affected by fluoroacetate is consistent with the hypothesis that alanine dehydrogenase provides a major route for alanine synthesis.

It should be noticed from Table 1 that the formation of glutamine is not inhibited by fluoroacetate. This supports the previous findings of Smith, Bassham, and Kirk (*2*), who concluded from kinetic data that glutamate was not a precursor of glutamine in *Chlorella*.

Evidence exists for the presence of the Krebs cycle in algae (*9*) but its relation to photosynthetic carbon dioxide fixation has not been clearly defined. In contrast to *Chlorella*, the present investigation indicates that in *Chlamydomonas* the synthesis of glutimate and aspartate during photosynthesis is closely associated with the Krebs cycle.

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