A. aegypti. This work has provided good evidence that it is the distension of the gut by the clotted blood which triggers a humoral chain of events culminating in maturation of the eggs.

We were interested in discovering if the absence of saliva affected the subsequent clotting of the blood in the mid-gut and if this would in turn affect egg development. The mid-guts of operated and blood-fed *A. stimulans* contain a blood clot which does not appear to be different, on examination under the microscope, from the clot formed in a normal mosquito. Moreover, the eggs develop to stages F and G (*4*), indicating that humoral activity has been initiated.

Recently we have been able to cut the salivary duct in *A. aegypti*, and experiments with mosquitoes of known age and diet should offer more accurate data. The results of the experiments are at present difficult to interpret in terms of the function of the salivary glands of mosquitoes. If saliva plays a part in digestion of the blood meal, this function may be reflected in the subsequent activities of the mosquito, rather than in the development of the current batch of eggs. It has been suggested (*5*) that the salivary glands of blood-sucking arthropods are now nonfunctional but remain as evidence of a plant-feeding ancestry. This seems unlikely in mosquitoes, in view of an apparent cyclical activity of the gland cells (*6*).

The presence of an anesthetic component is suggested by observations that the bites of *A. stimulans,* unaccompanied by saliva, are more painful than the bites of normal mosquitoes. Such a component would have considerable adaptive significance and might also be a factor determining the effectiveness of certain species as vectors of disease organisms. The success of insects as vectors has been viewed in this way by Herms (*7*; *8*).

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Residence Time of Dissolved Phosphate in Natural Waters

Abstract. Residence time varies from approximately 0.05 to 200 hours. Short residence times are indicative of depleted phosphate, active metabolic activity, or both. The turnover rate of phosphate is between 0.1 and 1.0 mg of phosphorus per cubic meter, per hour, regardless of phosphate concentration, except in biologically active systems where it is 1.0 to 20. The turnover rate of phosphate may be more important than the phosphate concentration in maintaining highly productive systems.

The use of $P^{32}O_4$ in studies of lakes has revealed dynamic equilibria between phosphate dissolved in the water and phosphorus in the plankton, benthic organisms, bacteria, sediments, and dissolved organic materials (*1–5*). The residence time of dissolved phosphate proved to be a matter of minutes (*3*). Estimates of the residence time of dissolved phosphate in the sea and in estuaries (Table 1) reveal a wide range of conditions under which phosphate equilibria may be established, with the residence time varying over several orders of magnitude.

Residence time and turnover rate of phosphate in freshly collected water were estimated in the laboratory. Aliquots of 1.5 liters of water were placed in 7-liter rolling bottles in a constant-temperature room, and the water was kept within ±2°C of its temperature at collection. The water was illuminated by flourescent lights with an intensity of 350 ft-ca. Sterile, carrier-free $P^{32}O_4$ was added to the water, and changes in radioactivity were measured at intervals until equilibrium was reached. Uptake of P^{32} by bacteria and plankton was measured by filtering aliquots of water through Millipore filters of 0.45-μ porosity and counting the activity of the dried filters. The residence time of phosphate was calculated by the method used in studies of lakes (*2–4*), but only the observations of Rigler (*3*) are comparable in the method of filtration.

Turnover rate is considerably less variable than residence time, and exceeds the range of 0.1 to 1.0 mg/m³ per hour only in biologically active systems, such as plankton blooms, salt marshes, and small lakes. Residence time is influenced both by the turnover rate and the concentration of dissolved phosphate. Therefore, a system having a short phosphate residence time may be impoverished in phosphate, as in the sea, or it may be unusually active biologically, as in algal blooms. When both conditions occur together, as in small lakes, the residence time becomes vanishingly short.

Measurements of the concentration of dissolved phosphate in natural waters give a very limited indication of phosphate availability. Much or virtually all the phosphorus in the system may be inside living organisms at any given time, yet it may be overturning every hour with the result that there will be a constant supply of phosphate for organisms able to concentrate it from a very dilute solution. Such systems may remain stable biologically and chemically for considerable periods in the apparent absence of available phos-

Table 1. Residence time, concentration, and turnover rate of dissolved phosphate in natural waters

Date	Location	Type of system	Dissolved phosphate			T (°C)
			Res. time (hr)	Concn. (mg atom P/m³)	Turnover (mg P/m³ per hr)	
9/28/54	Oiseau Lake, Ontario*	Small lake	0.06	0.003	1.6	
9/11 and 9/18/53	Toussaint Lake, Ontario*	Small bog lake	0.08	0.009	3.6	
9/17/54	Maskinonge Lake, Ontario*	Lake	0.4	0.012	1.0	
7/29/58	Salt-marsh creek, Georgia	*Kryptoperidinium* bloom	1.0	0.6	19	29
5/14/59	Altamaha River, Georgia	Nostocaceae bloom	1.0	0.1	3	25
7/18/58	30°53′N, 80°28′W	Continental shelf water	5	0.1	0.6	29
7/18/58	30°58′N, 80°01′W	Gulf Stream, surface	4	0.1	0.8	29
7/18/58	30°58′N, 80°01′W	Gulf Stream, 60 m	12	0.1	0.3	29
4/ 2/59	31°25′N, 81°05′W	Coastal sea water, surface	34	0.1	0.1	18
10/15/59	31°19′N, 81°10′W	Coastal sea water, surface	155	0.5	0.1	34
10/15/59	31°20′N, 81°13′W	Coastal sea water, surface	63	0.8	0.4	33
11/19/59	31°20′N, 81°13′W	Coastal sea water, surface	50	0.3	0.2	15
11/19/59	31°19′N, 81°11′W	Coastal sea water, surface	46	0.1	0.1	16
4/20/59	31°23′N, 81°17′W	Doboy Inlet	4	0.2	1.5	22
11/12/59	31°23′N, 81°17′W	Doboy Inlet	66	1.0	0.5	16
11/19/59	31°23′N, 81°17′W	Doboy Inlet	111	0.9	0.2	15
6/26/58	31°25′N, 81°18′W	Doboy Sound	37			
2/28/59	31°25′N, 81°18′W	Doboy Sound	50	0.6	0.5	12
7/17/59	31°25′N, 81°18′W	Doboy Sound	56	1.0	0.5	30
11/12/59	31°25′N, 81°18′W	Doboy Sound	39	1.0	0.8	15
11/19/59	31°25′N, 81°18′W	Doboy Sound	30	1.0	1.0	14
1/30/59	31°29′N, 81°16′W	Salt marsh (low tide)	49	3.0	2.0	15
10/12/59	31°29′N, 81°16′W	Salt marsh (low tide)	40	5.5	4.0	27
11/12/59	31°29′N, 81°16′W	Salt marsh (high tide)	169	1.1	0.2	15
11/12/59	Altamaha River, Georgia		13	0.2	0.5	14
9/14/54	Ottawa River, Ontario*		30	0.05	0.2	

* From Rigler (*3*). Rigler's observations have been converted from other units of measurement for convenient comparison.

phate. This suggests how it is possible for phytoplankton blooms to persist in water containing only a few hours' supply of dissolved phosphate. The observations presented here suggest that a rapid flux of phosphate is typical of highly productive systems, such as blooms, and that the flux rate is more important than the concentration of dissolved phosphate in maintaining high rates of organic production.

It would be of interest to learn what factors tend to stabilize the flux of phosphate over a wide range of phosphate concentrations and what factors induce a more rapid flux in certain circumstances (*6*).

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Biological Effects of a Chemical Mutagen, Diepoxybutane, on Tomato

Abstract. Tomato seeds were treated with three concentrations of 1:2, 3:4-diepoxybutane. Seedling mutations were induced in high amounts with all doses, but other biological effects in the treated generation including delayed germination, chlorophyll-deficiency sectoring in the first true leaves, and failure of plants to bear fruit, were found only with the highest concentration. Neither a lethal nor a partially lethal dose was applied.

Diepoxybutane has been demonstrated to be a highly effective mutagenic agent in barley by Ehrenberg and Gustafsson (*1*), in *Neurospora* by Kolmark and Westergaard (*2*), and in *Drosophila* by Bird (*3*) and others. Other biological effects, occurring simultaneously with the mutational effect of diepoxybutane in these biological materials, included sterility and lethality, which increased with increased chemical dose. However, in this study lethality was absent in all treatments, and sterility was restricted to only the highest concentration, while the mutational effect was observed with all doses of diepoxybutane applied.

Tomato seeds, 300 per treatment, of the highly inbred O18 strain of the Red Cherry variety of *Lycopersicon esculentum*, were treated with three concentrations of 1:2, 3:4-diepoxybutane—0.2, 0.5, and 0.8 percent—in aqueous solution and with distilled water. Preliminary investigations indicated the 50 percent lethal dose to be somewhere between 0.1 and 1.0 percent diepoxybutane when the seeds, presoaked for 24 hours in distilled water, were treated in the chemical for 1 hour under vacuum followed by 5 hours at normal pressure. The same conditions of treatment were followed throughout.

Table 1. Number of mutations recovered in the second generation following treatment of tomato seed with various concentrations of diepoxybutane, or (control group) with distilled water.

Treatment	No. of transplanted seedlings	No. of mutants recovered	Percent of plants producing mutants
Control (distilled H_2O)	69	0	0.0
0.2 percent diepoxybutane	110	5	4.5
0.5 percent diepoxybutane	104	4	3.8
0.8 percent diepoxybutane	40	7	17.5

Following treatment, the seeds were thoroughly washed and planted in soil. After 2 weeks the seedlings were transplanted to pots on a raised bench in a greenhouse maintained at 65°F. night temperature. The number of transplanted seedlings in each treatment were as shown in Table 1. The plants, trained to one main axis, were grown for 7 months and seeds from the first four fruit-bearing inflorescences were recovered separately.

For the determination of induced mutations the seeds from the first and last inflorescence of each plant were grown to the seedling stage of the second generation and screened for seedling abnormalities. Lines segregating for color, rate of growth, or morphological seedling abnormalities were grown to the third generation to determine the inheritance of the abnormalities recovered. Abnormalities segregating in a 3:1 ratio in the seedling stage of the third generation were classified as mutations.

Mutations were recovered from all three diepoxybutane treatments but none from the control lots. The number and percentage of mutations recovered from each diepoxybutane treatment are listed in Table 1. Only a single mutant type was recovered from any one treated plant, but the mutation could be found in either or both of the tested inflorescences of this plant. The majority of the mutants were chlorophyll-deficient types; in the remainder, rate of growth and morphological characteristics were affected.

Apparent pleiotropic effects on rate of growth and morphological development were characteristic of many of the chlorophyll mutants. Lethality in the seedling stage or sterility of most of the mutants prevented the recovery of the mutations in a homozygous condition. This fact might be an indication of chromosomal deletions or rearrangements in the mutants rather than true gene changes.

Biological effects in the treated generation, excluding the mutational effect, resulting from diepoxybutane treatment were found only with the highest concentration, 0.8 percent diepoxybutane. The seed treated with 0.2 and 0.5 percent diepoxybutane developed in all respects the same as the control. The mutational effect in seed treated with these two concentrations was the only observable difference from the seed treated with distilled water.

The three characteristic effects of the 0.8 percent dieoxybutane treatment in the treated generation included delayed germination, chlorophyll-deficiency sectoring in the first true leaves, and the production of fruitless plants.

While the control, 0.2 percent, and 0.5 percent diepoxybutane treatments yielded 90 percent germination of the treated seed within 10 days of planting, during the same period only 15 seeds, or 5 percent, in the 0.8 percent diepoxybutane treatment germinated. However, 3 weeks later germination had increased to 90 percent, thus demonstrating the failure to administer even a partially lethal dose.

All of the seedlings that germinated in the 0.8 percent diepoxybutane treatment exhibited chlorophyll-deficiency sectoring in the first true leaves. All other leaves developed normally.

In this experiment, sterility was determined by the number of plants failing to produce any fruit; a treated plant was considered fertile if it produced at least one fruit on any of the tested inflorescences. In the 0.8 percent diepoxybutane treatment, eight plants, or 20 percent, failed to produce fruit. In the other two chemical treatments together, only one plant, of the 0.2 percent diepoxybutane treatment, was fruitless. However, all of the plants which failed to produce fruit in the greenhouse on the main axis, when they were pruned back to the first leaves and set in the field, produced fruit on their axillary branches in all respects the same as normal plants.

The tomato appears in this experiment to respond differently from other biological materials to diepoxybutane