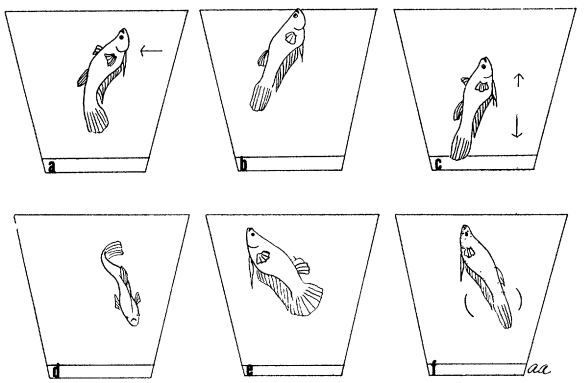

Fig. 1. The six diagrams illustrate the nine criterions or effects of LSD 25 upon the fighting fish, *Betta splendens*. Such effects are essentially exaggerated postures or caricatures of the normal fish.

1) Backward movements; accomplished almost entirely by pectoral fin movements (Fig. 1*a*).

2) Head up, body usually suspended in the vertical plane or some angle from the vertical. In maximum stage of narcosis the snout is kept at the surface. As effects begin to wear off, the fish sinks slowly below the surface until after 6 hr it might take a position 2 in. below the surface (Fig. 1*b*).

3) "Cartesian diver" effect. Treated fish sinks or rises very slowly in near-vertical plane without visible body movement except by means of pectoral fins (Fig. 1*c*).

4) "Barrel-roll" effect, change of position or location is by a peculiar rolling of the fish upon its long axis in the vertical plane (Fig. 1*a, f*).

5) "Trancelike" effect, motionless position maintained for minutes at a time at the peak of the narcosis. The "trance" is broken by a very brief change of position by means of a very slight stimulus. Succeeding "trances" become gradually shorter after cessation of treatment (Fig. 1*e*).

6) All movements of treated fish are slow and deliberate as compared with the typical swift and sudden movements of normal fish (Fig. 1*a, c, d, f*).

7) Treated fish exhibits a typical "kinking" in its body conformation, easily observed from above (Fig. 1*d*).

8) Lateral display, most commonly involving the ventral and dorsal fins, less usually the tail as well. This posture persists while fish is in trancelike state (Fig. 1*e*).

9) Pigmentation effects, best exhibited in juveniles. Immediate effect is darkening of basic body color. This fades slowly as recovery occurs (Fig. 1*f*).

Table 1 shows the manner in which the foregoing criterions may be used to follow the reactions of the fish to the drug. Note in the table a reaction of recovery that we have consistently observed. This reaction is a slow return to normal from the stuporlike state induced by the drug with coemplete recovery in the low dose range within a day and with the high dose range within a week. Although recovery usually occurs within a week, exposure to the drug has, in many cases, actually altered the social behavior. This will be described in a future paper. However, we should call attention to some of these aspects of behavior. For example, the lysergized fish can be aroused from a stupor by an attacking male and even counterattack. But after a brief battle, there is an immediate relapse into the stuporous state, showing many of the afore-mentioned nine criterions. Lysergized fish will respond rheotropically but not as effectively as untreated fish. All the effects have been observed in both sexes and in unsexed juveniles. Quantitative data are not yet available on the way weight, age, and dosage are related. The qualitative data hold for the species ranging in body length from 1 in. to full adult size, as studied at Cold Spring Harbor in the summer of 1954. Fish were obtained from various dealers and, consequently, were of heterogeneous genetic make-up.

In conclusion, we would like to point out that our technique provides a new bioassay method for LSD 25 and possibly other ergot drugs. For example, LSD 25 in urine and other body fluids is difficult to determine by chemical means. Preliminary experiments on urine show that *Betta* has essentially normal activity in spring water containing as much as 25 percent of urine for at least as long as 44 hr. This opens up the possibility not only of detecting LSD 25 in urine, but also of studying the urines of clinically schizophrenic patients for chemical agents occurring naturally, which might be the cause of clinical schizophrenia in man. It should be emphasized that not a single fish has been lost owing to the action of the drug, regardless of dosage used, even after the injection of 50 μg in the caudal musculature.

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Inhibition of Growth of Excised Tomato Roots by Desoxypyridoxin and Its Reversal by Pyridoxin

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Using microorganisms or animals as test material (*1, 2*) a number of analogs of pyridoxin have been found to act as antimetabolites. Similar results using test material from higher plants are scanty. Robbins (*3*) studied the specificity of pyridoxin in the nutrition of excised tomato roots and found that it could be replaced by either one of two acetoxy analogs. Two other analogs inhibited growth, but it was not shown that pyridoxin could prevent the inhibition. It has, however, been shown (*4*) that 4-desoxypyridoxin (DOP) inhibited the increase in nicotinic acid and ascorbic acid found in certain germinating pulse seeds

Table 1. The effect of supplying desoxypyridoxin and pyridoxin in various ratios on the growth of excised tomato roots.

Level of desoxypyridoxin	Level of pyridoxin	Ratio of analog to metabolite	Increase in length of main axis per root (mm)	Number of laterals per root	Total length of 10 basal laterals per root (mm)
30	10.0	3:1	91.6 ± 4.14* (9)†	32.3 ± 2.08	66.3 ± 4.96
300	100.0		102.2 ± 2.55 (6)	37.7 ± 2.51	40.2 ± 4.83
10	3.0	10:3	109.3 ± 2.86 (8)	39.6 ± 1.94	41.4 ± 4.15
100	30.0		109.4 ± 5.53 (7)	43.6 ± 2.89	52.0 ± 5.29
5	0.5	10:1	87.0 ± 5.15 (7)	26.9 ± 10.96	37.8 ± 3.24
10	1.0		100.7 ± 3.19 (7)	36.6 ± 3.05	44.1 ± 6.07
30	3.0		95.3 ± 2.98 (8)	34.6 ± 2.15	44.6 ± 2.96
100	10.0		85.7 ± 3.56 (7)	26.9 ± 1.50	51.7 ± 2.09
300	30.0		72.3 ± 3.53 (7)	23.6 ± 1.65	48.6 ± 4.05
30	1.0	30:1	35.1 ± 4.29 (7)	15.6 ± 1.73	60.9 ± 7.58
300	10.0		9.5 ± 0.53 (8)	7.3 ± 0.49	
30	0.5	60:1	10.8 ± 1.37 (8)	9.5 ± 0.78	
100	3.0	100:3	27.0 ± 4.42 (8)	14.0 ± 1.92	
1000	30.0		5.3 ± 0.89 (7)	5.9 ± 0.41	
100	1.0	100:1	4.7 ± 0.84 (6)	5.8 ± 0.16	
300	3.0		4.2 ± 1.08 (6)	5.5 ± 0.25	
	0.5		95.7 ± 4.99 (6)	32.8 ± 2.51	47.5 ± 8.17
	1.0		92.0 ± 3.68 (8)	31.1 ± 2.59	58.3 ± 6.16
	3.0		81.9 ± 6.96 (7)	31.0 ± 2.96	57.6 ± 9.06
	10.0		93.3 ± 4.99 (7)	33.9 ± 4.71	61.9 ± 7.15

* Standard error.
† Number of replicates.

and that the inhibition was reversed by pyridoxin. The type of antagonism between pyridoxin and DOP was not established.

The evidence in the literature (*1, 2*) shows that analogs of pyridoxin are not competitively antagonistic toward pyridoxin in animals or in microorganisms that do not require vitamin B_6 for growth. The experiment reported here was designed to determine whether this was the case using DOP with the higher plant material represented by cultures of excised tomato roots. The general techniques used are described elsewhere (*5*). The clone of excised roots used as source of inocula requires pyridoxin, pyridoxal, or pyridoxamine for growth. It is designated R5 (*6*). The inhibition of growth on addition of various levels of DOP to the basal medium plus thiamine and its reversal by pyridoxin are shown in Table 1. The standard concentration (1.0) of either pyridoxin or DOP was equivalent to $4.9 \times 10^{-7} M$ of the free base.

Inhibition by concentrations of DOP of 10, 30, 100, and 300 times the standard concentration was completely reversed by increasing the concentration of pyridoxin. Therefore, within the limits of the method, the antagonism can be considered competitive. However, when concentration of DOP was increased and ratio of analog to metabolite remained constant (10:1 or 30:1) the growth of main axis decreased. In accordance with the principles involved in inhibition analysis (*1*), such an effect on a competitive inhibition could be obtained if the competition occurred between products of the analog and metabolites as supplied in the medium.

The exact mechanism whereby analogs of pyridoxin inhibit growth and enter into competition with vitamin B_6 is not clearly understood. The subject is reviewed elsewhere (*7*), and some additional evidence from enzyme studies *in vitro* has been presented (*8*). The mechanism probably varies with the analog and with the organism. In some instances it may involve the conversion of DOP to DOP-phosphate, which then competes with pyridoxal phosphate or pyridoxamine phosphate. It has been shown (*5*) that excised roots of the clone used here utilize pyridoxal or pyridoxamine in place of pyridoxin. The results obtained here, therefore, are in agreement with the view that the antagonism between pyridoxin and DOP involves prior conversion to the phosphate.

The data for growth of laterals are not completely in agreement with those for main axis. Thus, at the five levels of DOP at which the ratio of analog to metabolite was 10:1, growth of laterals did not decrease with increase in concentration. An explanation of this discrepancy is hard to find but may involve the mechanism of the correlative influence of the main apex upon growth of laterals (*9*).

A final point of interest is the fact that growth of the main axis was significantly greater in the presence of DOP and pyridoxin in the ratio 10:3 than with any concentration of pyridoxin alone. This indicates a function of pyridoxin in some mechanism (or mecha-

nisms) which inhibits or restricts growth. Such a possibility is also indicated by the reduction in growth in length of the main axis obtained with pyridoxin at 3.0 times standard in the control mediums. This inhibitory effect of pyridoxin, at approximately this same concentration, has been observed in other experiments not yet published.

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Effect of Linear Energy Transfer on Radiation-Induced Chromosome Aberrations in *Tradescantia* Microspores[1]

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Comparative studies in which various types of ionizing radiations were utilized have provided evidence for a marked effect of ionization distribution (specific ionization) on the production of chromosome aberrations in *Tradescantia* microscopes. These studies have involved the use of different radiations—for example, x-rays and fast neutrons (*1*) and alpha particles (*2*)—to provide different patterns of ionization distribution. The recent availability of more powerful particle accelerators and improved exposure techniques have now made possible direct comparisons of the effect of linear energy transfer (rate of energy loss) utilizing single particle beams (*3*).

This paper presents the results of some experiments (*4*) carried out in the summer of 1948 with the 184-in. Berkeley cyclotron. The experiments must be considered to some extent preliminary, since in certain instances, especially with alpha-particle exposures, the quantitative data are not as extensive as is ordinarily desirable. Since it has not yet proved feasible to expand these observations, as was originally contemplated, it seems desirable to record the data and conclusions obtained to date.

Deflected beams of 190-Mev deuterons and of 380-Mev alpha particles were used. The particles had nearly parallel trajectories, and the radiation field covered an area of about 1 in. in diameter. By placing absorbers in front of the plant, the beam energy could be lowered. Data are reported here for high-energy deuterons having linear energy transfer (LET) of 0.73 kev/μ tissue (position I); deuterons, slowed by 1842 mg/cm^2 of aluminum, having a LET between 5 and 30 kev/μ at the neighborhood of the Bragg ionization peak (position III); high-energy alpha particles with LET of 2.9 kev/μ. Buds from fresh inflorescences were immersed in water and exposed near the center of the radiation field, with the axis of the buds parallel to the beam. Because of the variations of the size of buds and position of the pollen grains within, both the dose and LET of the low-energy deuterons may be in considerable error. Exposure normally took less than 1 min, and dosimetry was done by means of parallel plate ionization chambers (*3*).

Following irradiation, inflorescences were placed in containers of water and maintained at room temperature. Slides were prepared by the acetocarmine smear technique at 24 hr following radiation exposures for an analysis of chromatid aberration frequencies.

The results reported here are confined to isochromatid and chromatid aberration types. The first comparison was obtained in exposures of inflorescences to deuterons of two different energies. The results are given for isochromatid aberrations in Table 1 and Fig. 1 and for chromatid aberrations in Table 1. Standard errors have been calculated as described by

Table 1. Frequencies of isochromatid and chromatid aberrations induced by deuterons in *Tradescantia* microspore chromosomes. Slides made 24 hr after irradiation. (Position I, low LET; position III, high LET. See text.)

Expt.	Dose (rep)	Position	No. of cells	Isochromatid aberration	Isochromatids per cell	Chromatid aberration	Chromatids per cell
1	36	I	675	52	0.08 ± 0.01	34	0.05 ± 0.01
1	70.7	I	558	68	.12 ± .01	29	.05 ± .01
2	24.2	I	600	25	.04 ± .008	15	.025 ± .006
2	46.7	I	300	18	.06 ± .01	12	.04 ± .01
2	97	I	250	56	.22 ± .03	24	.10 ± .02
2	187	I	50	22	.44 ± .09	14	.28 ± .07
1	48.2	III	152	76	.50 ± .06	34	.22 ± .04
2	11.0	III	250	33	.13 ± .02	8	.03 ± .01
2	22.4	III	350	57	.16 ± .02	24	.07 ± .014
2	44.5	III	300	100	.33 ± .03	27	.09 ± .02
2	65.7	III	210	131	.62 ± .05	48	.23 ± .03