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Effect of thalidomide on *Tribolium confusum* Duval

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THE association of human fetal abnormalities with thalidomide has led to an increasing amount of experimental work on various laboratory animals during the past two years. In the present communication we have investigated the effect of thalidomide on *Tribolium confusum*. This insect was selected for the study because of its short life span and distinct life stages.* In addition, such an investigation might be of interest for comparative purposes.

* Life cycle of *T. confusum* may be divided into five well-defined stages: (i) embryonic (6 days); (ii) larval (14 days), a growth phase, duration of which is considered a criterion for growth; (iii) pre-pupal (3 days); (iv) pupal (6 days); (v) adult (up to 2 years).

METHODS

The methods of rearing *T. confusum* were those of Chaudhary and Lemonde.[1] For studying the effect of thalidomide on growth, fecundity, and fertility, varying amounts of this drug were introduced into a basic diet consisting of whole wheat flour supplemented with 5% dried brewer's yeast to ensure adequate supply of vitamin B complex. The basic diet alone served as the control diet.

For each of the diets used, 50 larvae up to 15 hr old were divided into 5 lots of 10 each. These were deposited in vials measuring 1·5 in. dia. by 2·5 in. high and containing 3 g of diet each. The vials were then kept under constant conditions of temperature (28° ± 1°) and relative humidity (70% ± 5%) in a controlled chamber. The number of days required by the larvae to transform themselves into pupae were recorded.

Fifteen pairs of newly emerged adults were placed on each of the diets. The adults were allowed to mate freely and the eggs laid during the first 60 days were collected at 5-day intervals. In order to verify the existence of infertile eggs, if any, that might have been produced under the influence of thalidomide, a separate experiment was run under identical conditions except that the adults which were initially given each diet were removed after 30 days. The eggs laid during these 30 days were allowed to hatch into larvae which subsequently became pupae. During the following 30 days, the pupae appearing in each of the diet vials were collected at 5-day intervals.

For investigating the effect of the drug on the insects of the second generation, 15 pairs of adults from each of the diets were fed a corresponding diet. The rest of the experimental procedure was carried out as for the first-generation insects.

From each diet, 5 representative samples of the pupae taken at suitable intervals and under similar experimental conditions were sexed and weighed. Oxygen consumption of the pupae from the various diets was estimated by means of a Warburg constant-volume respirometer.[2] According to methods described by Chaudhary and Lemonde,[3] the activity of acid and alkaline phosphatase was estimated in the whole insects fed on different concentrations of thalidomide in the diets.

RESULTS AND DISCUSSION

Effect on growth. Unlike mammals[4–9] and birds,[10–13] we noted no visible malformations caused by thalidomide during any stage in the life cycle of *T. confusum*. Lucey[14] observed that treated monkeys produced no live births, and fetal resorption was noticed in thalidomide-administered rats by several workers.[4, 5, 15] We found that thalidomide retarded the growth in *T. confusum* slightly (Table 1). This drug has also been reported to cause inhibition in the growth of certain protozoa.[16]

TABLE 1. EFFECT OF THALIDOMIDE ON GROWTH OF *T. confusum*

Thalidomide in diet (%)	Number of pupae from 50 larvae ♂	♀	Larval period (days)* Mean ± σM	Range
0 (control)	23	25	17·29±0·001	16–19
0·001	30	19	17·57±0·002	17–19
0·01	25	20	17·71±0·001	17–19
0·1	22	25	18·01±0·009	17–20
1·0	22	21	19·97±0·050	18–22
4·0	22	20	20·00±0·068	19–23

* No difference was observed between the male and female larval periods.

$M = fm/N$ where M = mean larval period in days; m = n'th day of the experiment; f = number of pupae on the n'th day of the experiment; N = total number pupae.

$\sigma M = \sqrt{[f(M - m)^2/N^2]}$.

Effect on fecundity. Thalidomide had, however, a marked inhibitory effect upon fecundity in *T. confusum* (Table 2). Reserpine[17] and some amino derivatives of phenothiazine[18] were reported earlier to have produced inhibition at the level of fecundity in the same insect.

TABLE 2. EFFECT OF THALIDOMIDE ON FECUNDITY OF *T. confusum*

Thalidomide in diet (%)	Eggs produced	
	Weight/100 eggs (mg)	Total number*
0 (control)	4·82	9,017
1·0	5·00	8,772
4·0	4·90	7,296

* Total number of eggs (both fertile and infertile) laid during 60 days represents an index of fecundity.

Effect on fertility. Thalidomide also caused the production of infertile eggs (Table 3). Our result seem to be in conformity with the findings of Shorb *et al.*[19] who showed that White Leghorn hens fed on thalidomide in total doses of 70 to 1,700 mg per hen, increased the total number of infertile eggs.

TABLE 3. EFFECT OF THALIDOMIDE ON FERTILITY OF *T. confusum*

Thalidomide in diet (%)	Total number of pupae*	
	First generation	Second generation
0 (control)	1,971	1,939
0·001	2,062	1,871
0.01	2,161	1,925
0.1	2,261	1,904
1·0	1,650	1,721
4·0	1,366	1,361

* Total number of pupae represents the total number of fertile eggs laid during 30 days and therefore serves as an index of fertility.

Effect on fresh weight. As for the pupal fresh weight, we observed hardly any difference between the normal and the thalidomide-fed insects (Table 4). Finally, no major variation occurred between the individuals of the two sexes.

TABLE 4. EFFECT OF THALIDOMIDE ON PUPAL FRESH WEIGHT OF *T. confusum*

Thalidomide in diet (%)	Weight per 100 pupae (mg)*			
	First generation		Second generation	
	♂	♀	♂	♀
0 (control)	259·5	290·8	252·4	287·6
0·001	253·9	282·7	256·4	282·2
0·01	252·4	280·5	249·3	275·5
0·1	255·5	285·9	262·2	297·2
1·0	236·0	280·5	269·5	298·3
4·0	235·6	283·8	254·9	282·1

* The weights are of the pupae from the preceding experiment (Table 3).

Effect on oxygen consumption. Studies of oxygen consumption in *T. confusum* revealed no differences between the thalidomide-treated and control insects. This seems to be in agreement with the findings of Fabro *et al.*[20] who investigated the oxygen uptake of homogenized 15-day-old rabbit fetuses. As pointed out by these authors, thalidomide might not, therefore, affect the energy-yielding processes. Frank *et al.*,[16] however, counteracted thalidomide inhibition in some protozoa by nicotinic acid and vitamin K, suggesting that the mechanism of toxicity might be an interference in cellular oxidation. Unlike reserpine,[17] this drug had no influence on the spontaneous activity of the insect. In this respect its effect may be compared to that in higher animals. Taussig[21] mentioned that thalidomide did not induce sleep in animals, whereas it acted as a sedative in human beings.

Effect on phosphatase activity. Both acid and alkaline phosphatase activity in the pupae from diets containing up to 1% of thalidomide showed no difference from those on the control diet.

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Dissociation of reserpine-induced depression of spontaneous motor activity and release of brain serotonin in rats

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THE mechanism of the central depressant action of reserpine is not completely settled after almost ten years of investigation. A great body of evidence has been presented in favor of a concept that reserpine-induced release of serotonin (5-hydroxytryptamine, 5-HT) from its binding sites in the brain causes the tranquilizing action of the drug.[1–15] In opposition to this theory some arguments have been made in support of a role for the release of brain norepinephrine in the depressant action of reserpine.[16, 17]