the oxygen concentration of the medium. This was accomplished by placing the same number of *Hydra* in each of a series of half-filled serum bottles hermetically sealed with rubber stoppers through which varying amounts of air could be withdrawn by means of a syringe and hypodermic needle. After 3 wk without feeding at 20°C, during which time no asexual increase occurred, testes were observed on 90 percent of the *Hydra* in the serum bottle whose atmospheric pressure had been reduced 20 percent to 608 mm of Hg. No sexual forms were observed in control cultures exposed to the air in shallow vessels or in serum bottles evacuated below 388 mm pressure.

These results were confirmed by keeping shallow cultures (4 mm deep) of *Hydra* in Petri dishes placed within vacuum desiccators where the partial pressure of oxygen could be adjusted to any desired level by means of a water aspirator attached to a mercury manometer (*18*). Testes were observed in 17 days at 23°C in cultures fed twice a week and maintained at 610 mm of Hg, while none were observed in cultures maintained at either 760 mm of Hg or below 532 mm of Hg pressure.

Additional evidence was obtained by allowing a clone of *Hydra* to increase asexually under uniform conditions of temperature, stagnation, and nutrition until the crowding had increased to a point, critical for each vessel and depth of medium, where the average oxygen concentration had fallen to about 6–7 mg/l O_2. Once this critical density had been reached, further asexual increase was inhibited and sexual forms appeared. Since routine oxygen determinations are not required in this method, it is convenient and has been used successfully with both male and female clones (*19*) at 20°, 25° and 30°C.

When the resulting sexual *Hydra* were returned to fully aerobic conditions in shallow Petri dishes, they did not revert back to the asexual state but continued to develop spermaries and ovaries at the same time that they budded asexually. Since these buds spontaneously became sexual within a few days, a permanently "sexual" culture became possible, one that has maintained a high degree of sexuality for more than 5 mo under the identical culture conditions used in maintaining the usual "asexual" cultures (*13*). This apparently permanent modification of the phenotype by environmental conditioning represents a further example, it would seem, of *Dauermodifikationen* as recently discussed by Sonneborn (*20*). Its reversibility was demonstrated by reducing the oxygen tension to about 2.5 mg/l O_2 using the vacuum desiccator method described. Under these conditions, the growth of sexual organs was specifically inhibited within about 10 days, while asexual reproduction by budding continued as before.

These results appear to indicate that the primary stimulus that induces sexual differentiation in *Hydra littoralis* is a critical lowering of the oxygen concentration of the medium. This state of partial anaerobiosis may be induced in turn by external conditions such as are found during winter anaerobiosis in ponds (*21, 22*) or internally within a clone by simple crowding. Whether these factors are involved in the environmental induction of sexual differentiation in other species of *Hydra, Volvox,* sponges, rotifers (*2*), Cladocera (*12*), protistans such as the ciliate previously mentioned (*11*) or the malaria plasmodium (*23*), or even in aggregating myxamebas (*24*), and so forth, only further work can determine.

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Toxicity of Peptides of Thienylalanine for Rats

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Certain peptides containing the amino acid analog, β-2-thienylalanine, have been reported to inhibit the growth of bacteria (*1*). It has been further reported that a proteolytic enzyme, carboxypeptidase from beef pancreas, hydrolyzed carbobenzoxyglycyl-β-2-thienylalanine *in vitro* at the terminal peptide bond to liberate β-2-thienylalanine (*2, 3*). It therefore seemed of interest to determine the effect of peptides containing thienylalanine upon the growth of rats, with the hope of gaining some evidence concerning the ability of enzymes of the animal to hydrolyze the peptides *in vivo*.

Several workers have reported the toxicity of β-2-

Table 1. Inhibition of the growth of rats by β-2-thienylalanine and its derivatives; reversal of inhibition with phenylalanine.

Rat*		Supplement to basal diet (%)	Initial weight (g)	Final weight (g)	Days	Average gain per day (g)
17	2	β-2-Thienylalanine	130	115	11	− 1.4
	2	β-2-Thienylalanine *plus* 1 phenylalanine	115	130	10	1.5
30	2	β-2-Thienylalanine	214	136	24	− 3.2
	2	β-2-Thienylalanine *plus* 1.6 phenylalanine	136	162	10	2.6
10	1.6	Glycyl-β-2-thienylalanine	137	117	14	− 1.4
4	2	Glycyl-β-2-thienylalanine	195	159	5	− 7.2
38	2	Glycyl-β-2-thienylalanine	279	218	20	− 3.0
	2.7	Glycyl-β-2-thienylalanine *plus* 1.6 phenylalanine	218	230	4	3.0
41	1	Chloroacetyl-β-2-thienylalanine	68	70	8	0.25
3	2	Chloroacetyl-β-2-thienylalanine	115	91	11	− 2.2
	2	Chloroacetyl-β-2-thienylalanine *plus* 1 phenylalanine	91	80	4	− 2.75
	2	Chloroacetyl-β-2-thienylalanine *plus* 2 phenylalanine	80	72	4	− 2.0
37	2	Chloroacetylphenylalanine	124	91	11	− 3.0
	2	Chloroacetylphenylalanine *plus* 1 phenylalanine	91	86	4	− 1.25
	2	Chloroacetylphenylalanine *plus* 2 phenylalanine	86	77	4	− 2.25
37a	1	Chloroacetic acid	185	116	5	−14.0
7	2.1	Carbobenzoxyglycyl-β-2-thienylalanine	136	128	14	− 0.6
47	4.2	Carbobenzoxyglycyl-β-2-thienylalanine	294	195	20	− 5.0
	4.2	Carbobenzoxyglycyl-β-2-thienylalanine *plus* 1.6 phenylalanine	195	186	6	− 1.5
20	1	Carbobenzoxyglycine	200	223	4	5.8

* Other animals were used in each study, but these are reported as typical.

thienylalanine for rats and the ability of phenylalanine to reverse the toxicity (*4, 5*). The work (*6*) reported here shows that some peptides containing thienylalanine are as toxic as free thienylalanine; the toxicity of some of these peptides is relieved by phenylalanine. In the cases where the peptide toxicity is identical with that of free thienylalanine and is relieved by phenylalanine, it seems reasonable to suggest that the toxicity of the peptide results from the formation of thienylalanine by enzymatic hydrolysis of the peptide. On the other hand, in the cases where the thienylalanine derivatives are not toxic or where the toxicity is not reversed by phenylalanine, it seems reasonable to conclude that such peptides are not hydrolyzed to any appreciable extent to produce thienylalanine.

Table 1 summarizes the effect of the compounds studied upon the growth of rats. It is evident that glycyl-β-2-thienylalanine was similar to free β-2-thienylalanine in its toxicity for the rat; the toxicity was reversed by phenylalanine. Apparently, glycyl-β-2-thienylalanine was hydrolyzed by some peptidase of the rat to liberate the free amino acids, glycine and β-2-thienylalanine.

Chloroacetyl-β-2-thienylalanine and carbobenzoxyglycyl-β-2-thienylalanine inhibted growth of the rats, but the inhibition was not reversed by phenylalanine at the levels tested. This indicates that the toxicity was not the result of the formation of free thienylalanine but might be caused by the peptide itself or by some other product of hydrolysis. Enzymatic hydrolysis of carbobenzoxyglycyl-β-2-thienylalanine at the terminal peptide bond would produce carbobenzoxyglycine and β-2-thienylalanine. Since carbobenzoxyglycine was not toxic, and since the toxicity of this peptide was not reversed by phenylalanine, the conclusion is that carbobenzoxyglycyl-β-2-thienylalanine was not hydrolyzed to any appreciable extent by the rat.

The toxicity of chloroacetyl-β 2-thienylalanine and chloroacetylphenylalanine were of the same order, and free phenylalanine did not reverse the toxicity. Since chloroacetic acid was found to be highly toxic, it was concluded that the chloroacetyl amino acids might be hydrolyzed to produce chloroacetic acid. This conclusion is supported by the unpublished finding that carboxypeptidase can hydrolyze chloroacetylphenylalanine and chloroacetylthienylalanine *in vitro* at the same rate.

These studies were conducted upon male rats fed a diet of commercial rat chow. The compounds tested were uniformly mixed with the chow by grinding. In preliminary experiments, it was found that a diet containing 2 percent thienylalanine in the commercial chow inhibited growth to the same extent as that reported by Ferger and Du Vigneaud (*4*) using 2 percent thienylalanine in a synthetic diet.

All compounds tested were of the racemic variety.

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