The latter system became completely inactivated upon exposure to the long-chain alcohol. This held true even when all the precautions necessary for vigorous succinic oxidase action (*9*) were strictly observed and the activity was measured by two different methods (*17, 18*). This finding is in accord with recent experiences and others (*19*) and certainly argues against the postulation of a succinic oxidase-like complex as an explanation for some of the past difficulties in handling monoamine oxidase.

Irrespective of this theoretical consideration, the profound change in the physical status of monoamine oxidase consequent to the treatment outlined in foregoing paragraphs has some practical implications. It should now be feasible to obtain preparations of monoamine oxidase pure enough for physiological and therapeutic investigation (*4*).

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Reversible Induction of Sexual Differentiation in *Hydra*

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The chemical nature of the stimulus that induces sexual differentiation in *Hydra* has long been of interest to investigators. Since sexual differentiation in these animals is not an obligatory phase in their life-cycle, which can be continued indefinitely by the asexual mechanism of budding if necessary, some environmental stimulus reversibly controls their sexual differentiation so that the interstitial cells of the ectoderm develop into spermaries and ovaries at certain times and not at others (*1–4*).

* With the technical assistance of John H. Kirhoffer.

Previous workers have reported that sexual specimens of *Hydra,* usually found in nature in the fall or winter, appear from time to time in laboratory cultures without apparent cause (*5*). Descriptions of the inducing circumstances have mentioned four variables: changes in *temperature,* both upward (*4, 6*) and downward (*1–3, 5*); changes in the degree of *stagnation* of the cultures (*7, 8*); changes in the state of *nutrition* varying from starvation to overfeeding (*9, 10*); and changes in the degree of *crowding* of the cultures. It is interesting to note that this last factor has been observed to be associated with sexual differentiation in the ciliate *Glaucoma scintillans* (*11*) as well as in Cladocera (*12*). No common mechanism by which these different variables may operate has yet been proposed.

In a recent report from this laboratory, it was noted that *Hydra* grown under controlled conditions increased asexually in strictly logarithmic fashion (*13*). Subsequently studies have shown that the rate of this logarithmic increase is a function of five variables (*14*) when, and only when, the cultures were grown in very shallow (4 mm) culture in Petri dishes. The necessity of maintaining highly aerobic conditions if reproducible results (± 5 percent) were to be obtained was especially apparent with cultures grown above room temperature, allowed to stagnate for days in the same water, heavily fed with living crustaceans or densely crowded. Since these same conditions had been described as associated with the induction of sexual differentiation in *Hydra,* it suggested that the actual stimulus might consist of a critical lowering of the oxygen tension of the medium.

Direct determination of the oxygen tension presented a problem at first, because the Winkler method of measuring dissolved oxygen requires water samples as large as 250 ml, while its microadaptations are unduly time consuming. Fortunately it was found possible to devise a colorimetric method for 0.5-ml samples that could be completed in about a minute (*15*). The availability of this new method made it possible to observe the oxygen concentration in all cultures of *Hydra* and especially in those that differentiated sexually when maintained under a variety of conditions of temperature, stagnation, nutrition, and crowding. It was found without exception in more than 30 cases that the oxygen concentration in such sexual cultures was reduced below 8 mg/l O_2 (*16, 17*), but not below about 4 mg/l O_2. The most effective concentration, in terms of both rapidity and percentage of sexual forms induced, seemed to be about 6–7 mg/l O_2. Highly aerobic cultures (> 8 mg/l O_2) continued to bud asexually, while anaerobic ones (< 1 mg/l O_2) neither budded nor developed sexual organs. Experimental cultures maintained between 5 and 8 mg/l O_2 by the daily addition of appropriate amounts of fully aerated water developed sexual specimens repeatedly within about 14 days at room temperature.

Evidence that neither temperature, stagnation, nutrition, nor crowding were involved *per se* was obtained by holding these factors constant and varying

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-