References and Notes

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Preparation of Soluble Monoamine Oxidase

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Since its discovery in 1928 (*1*), monoamine oxidase (*2*) has stubbornly resisted various attempts at purification (*1, 3, 4*). An explanation for this behavior emerged from the demonstration of the enzyme's intimate adherence to the cytoplasmic particulate elements (*5*). Various efforts were subsequently made to release this system into solution by means of disruption of these bodies. The result was either loss of activity or the production of smaller microscopic particles to which in turn the enzyme was attached (*6*). Such experiences strengthened the earlier opinion that "monoamine dehydrogenase" was part of a complex of several enzymes acting in unison or in sequence (*4, 7*), in a fashion parallel to that postulated in the case of succinic dehydrogenase (*8, 9*).

In order to achieve dissociation of this labile deaminase from the insoluble cytoplasmic residue in liver dispersions, some extraction procedures were reinvestigated (*10*). Morton's method (*11*) yielded an inactive aqueous phase with activity still bound to the tissue fragments that were drawn into the butanol. Extraction with ethyl ether merely forced the activity-rich particles to align themselves at the interphase, while mixtures of ether and alcohols caused irreversible inactivation, even in the cold. On screening a number of surface active agents, however, a surprising tolerance of this oxidase toward many commercial soaps and detergents became apparent. Three of these agents actually caused enhancement of the deamination rate (*12*) of tyramine by rat and rabbit liver homogenates in phosphate buffer. The increment of activity, in the presence of isooctylphenoxypolyethoxyethanol (*13*), polyoxyethylenelauryl alcohol (*14*), and Alconox® ranged in one experiment from 60 to 25 percent, in that order.

Isooctylphenoxypolyethoxyethanol, in addition to increasing the average reaction velocity more effectively than the others, also produced a striking translucency of the homogenates. Maximal clearing occurred as rapidly as 60 sec after addition of the alcohol. The performance and the appearance of the homogenates remained thereafter virtually unchanged, even after they had been stored for 3 days at room temperature. This long-chain alcohol was therefore used in more detailed investigation.

Variation of detergent concentration from 1 to 70 percent changed very slightly either the degree of clearing or the activity of a 20-percent rabbit liver homogenate. Routine use of a 5-percent (V/V) concentration was arbitrarily adopted for all subsequent tissue preparations.

Centrifugation of the treated homogenates at 2000 *g* for 30 min yielded a small volume of sediment that was poor in monoamine oxidase activity, while brisk activity was demonstrable in the supernatant. The untreated homogenates presented, as was expected (*5*), the opposite distribution, with the voluminous sediment containing almost all the activity. The picture was not significantly changed when centrifugation at 144,000 *g* (calculated for the bottom of the tubes) was allowed to proceed for 180 min. This higher centrifugal force did cause the accumulation of a waxy material on top of the sparkling clear supernatant. This waxy layer was poor in activity and was discarded in subsequent experiments.

Clear supernatants were centrifuged at 144,000 *g* in some experiments for several hours at about +4°C (*15*), followed by lowering of the temperature, without changing the centrifugal force. The contents of the tubes became solidified and were cut into several segments. Assay of fractions secured in this manner showed that a slow sedimentation of the enzyme had taken place. After removal of a firm, pasty pellicle of heavy, inactive material, the lowest segments had 25 percent higher activity than the uppermost.

The specific gravity of nonfractionated, active supernatants was of the order of 1.016 to 1.018. Sucrose solution, the medium widely employed in 0.88*M* concentrations for centrifugal isolation of intracellular components, had a specific gravity of 1.120. It has already been established (*11*) that particles of microsome size are sedimented out of this medium following centrifugation at 41,000 *g* for 2 hr. It would seem doubtful, therefore, whether any such submicroscopic granules could have remained in suspension following our protracted centrifugation at higher speeds, in a medium of lower specific gravity, and in the presence of phosphate buffer, which is known to cause these particles to aggregate. Furthermore, it was also demonstrated that the liver microsomes disintegrate on exposure to the detergent: the silky appearance of suspensions of isolated microsomes (*16*) was instantly lost on exposure to this agent, and an orange-red color appeared, which was due possibly to the release into solution of cytochromes. Recentrifugation showed little discernible sediment. Similar results were obtained in the case of washed rat liver mitochondria.

Other tissue enzymes, as well, were brought into solution by this procedure. Assays for acid and alkaline phosphatase, diamine oxidase, D-amino acid oxidase, and histaminase showed high activity in liver extracts of this type. Our experience with succinic oxidase, on the other hand, was in striking contrast.

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