(till 4 min) which then ceased. No tryptophan uptake was observed with cycloheximide-treated cells. These results led us to devise other experiments in a first attempt to distinguish between repression and feed-back inhibition. Ammonium citrate-grown cells were divided into 3 parts; 2 flasks (experiment 1 and 2) were supplemented with tryptamine and incubated for 0.5 h; tryptamine was removed from 1 culture by centrifugation and the cells were washed by subsequent centrifugation. These washed cells were transferred to ammonium citrate medium for additional 0.5 h (experiment 2). The third culture (experiment 3) served as control. 0.5-h-tryptamine supplemented culture (experiment 1) and the other 2 cultures were assayed for tryptophan uptake. The results in the figure indicated that no tryptophan was transported into either culture supplemented with tryptamine in contrast to unsupplemented control (experiment 3).

It can be concluded from the above results that the inability of tryptamine-grown cells for the uptake of tryptophan was caused by a severe feed-back inhibition of tryptophan uptake system, and not by repression of the formation of an uptake or transport component. Similar observations were reported in the case of glutamate transport inhibition by glutamine in Synchococcus cedrorum[9].

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Occurrence of sepiapterin deaminase in the normal type silkworm, Bombyx mori

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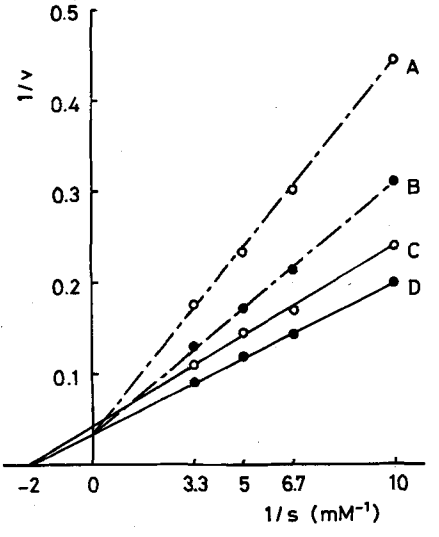
Summary. Sepiapterin deaminase was prepared from the normal strain of the silkworm, Bombyx mori. After inhibition experiments, this enzyme was found to have the same properties as that isolated from the lemon mutant strain. Several new inhibitors and their K_i values are described for the deaminase.

Sepiapterin deaminase was discovered in fat bodies of the lemon mutant strain of the silkworm, Bombyx mori[2]. Following this, the enzyme was purified from the integument of the same strain of silkworm and some of its properties were described[3]. Pterin and xanthopterin were shown at that time to be competitive inhibitors of the enzyme. The present paper reports the further study of the deaminase, in particular, the occurrence of reduced levels of the enzyme in the integument of the normal type silkworm, the mechanism of inhibition of additional inhibitors, and a comparison of the deaminase in the 2 types of silkworm, as well as with other pteridine deaminases.

Crystalline sepiapterin was obtained by the previously described method[4]. Other reagents were obtained from commercial sources. Sepiapterin deaminase was purified from both the normal type and the lemon mutant strains of Bombyx mori[3]. Data on the process of purification of the enzyme from the normal type silkworm is presented in table 1. The activity of the enzyme in crude extracts of normal type silkworm integument was from $^1/_{10}$ to $^1/_3$ less than that found with the lemon mutant strains.

Determination were made of the effect of several inhibitors on the deaminase prepared from both the normal type and lemon mutant strains of Bombyx. Reaction mixtures contained the following components in 1 ml of solution: potassium phosphate buffer, pH 8.0, 100 μmoles;



Double reciprocal plots of velocity versus sepiapterin concentration in the presence of inhibitors. Reaction mixtures are described in the text. Velocity is in terms of nmoles of sepiapterin decomposed per min. Plot A) 1×10^{-3} M 8-azaguanine present; B) 5×10^{-5} M amethopterin present; C) 5×10^{-4} M p-chloromercuribenzoate present; D) no inhibitor present.

Table 1. Summary of purification of sepiapterin deaminase from normal type silkworms

Step	Volume (ml)	Total activity (units)	Total protein (mg)	Specific activity	Yield
Crude extract	353	296.5	4006.6	1	100
Ammonium sulfate	42.5	416.5	850.0	6.6	140.4
DEAE-cellulose column	22.0	330.0	55.2	80.8	111.3
First hydroxylapatite column	30.0	67.5	9.07	100.5	22.8
Second hydroxylapatite column	6.7	28.5	0.58	658.1	9.6

Table 2. Inhibition of sepiapterin deaminase (%)

Compounds	Concentrations 10^{-2} M	10^{-3} M	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M
KF	72.0	18.6				
p-Chloromercuri-benzoate		80.0	11.8	0		
8-Azaguanine		51.3	17.3	0		
Phenyl methyl sulfonylfluoride		32.0	6.4	0		
Amethopterin			76.0	41.5	6.4	0
Aminopterin			83.2	14.3	7.9	0

sepiapterin, 0.4 μmoles; 3.5 units of enzyme, and inhibitor as described. After incubation at 25 °C for 10 min, 0.5 ml of 0.3 N NaOH was added and the decrease in absorbance at 475 nm was determined as previously described[3]. Sodium azide, KCN, monoiodoacetic acid, dinitrophenol, propionic acid, melamine and EDTA were not effective as inhibitors at a final concentration of 10^{-3} M for either type of deaminase preparation. Substances which were effective as inhibitors are listed in table 2. These compounds produced the same degree of inhibition with both type of enzyme preparation, thus indicating that the deaminase activity found in the 2 strains is due to 1 protein. The susceptibility of the enzyme to p-chloromercuribenzoate shows that sepiapterin deaminase differs from rat liver pterin deaminase[5] and Bombyx mori isoxanthopterin deaminase[6]. With 17.5 units of purified sepiapterin deaminase, isoxanthopterin deaminase activity was estimated[6], neither ammonia nor product 7-oxylumazine was detected. These data prove that the 2 enzymes differ from each other. Since bacterial pterin deaminase is inhibited by KF at a concentration of 3×10^{-5} M, it too is distinct from silkworm sepiapterin deaminase.

Lineweaver-Burk plots of normal and inhibited deaminase activity are shown in the figure. It can be seen that amethopterin and 8-azaguanine are competitive inhibitors of the enzyme, while p-chloromercuribenzoate is a non-competitive inhibitor. From the figure, K_i values for the substances were calculated as follows: amethopterin, 1.9×10^{-5} M; 8-azaguanine, 6.7×10^{-4} M; p-chloromercuribenzoate, 2.4×10^{-3} M.

The decreased concentration of sepiapterin deaminase in the integument of the normal type silkworm is noteworthy. In the normal type silkworm, it is probable the sepiapterin is converted to tetrahydrobiopterin via dihydrobiopterin. In the lemon mutant strain, a lack of sepiapterin reductase results instead in the accumulation of sepiapterin. The excess sepiapterin is excreted after deamination by sepiapterin deaminase, an action which produces a compound which is more water-soluble than sepiapterin itself. The high specific activity of the deaminase in malpighian tubules[3] supports this possibility.

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Large scale preparation of calf liver nuclei by continuous flow centrifugation

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Summary. Large scale purification and preparation of calf liver nuclei was accomplished by high speed centrifugation of a fraction enriched in nuclei ('nuclear homogenate') through 1.8 M sucrose by means of a Beckman CF-32 Ti continuous flow rotor. In comparison with methods involving the use of conventional high capacity rotors, larger volumes of homogenate could be processed. This method was used to prepare nuclei from calf liver for the preparation of DNA-dependent RNA polymerases. The use of continuous flow ultracentrifugation avoids time-consuming manipulations, thus allowing handling of large quantities of tissue.

High-density sucrose centrifugation is one of the most effective methods for preparing nuclei of animal cells free of contamination from other subcellular particles[3–5]. This point becomes of crucial importance when investigating enzymes present in multiple forms which are located in different subcellular particles. The low content in normal conditions of such enzymes often requires an enrichment of the selected subcellular particles from large quantities of starting tissue material. Whilst studying DNA-dependent RNA polymerase from different calf organs, we developed a method of preparing nuclei from calf liver in a high yield with a good degree of purity, using a continuous flow ultracentrifugation.

Experimental. Sucrose and $MgCl_2$ were reagent grade. A Beckman CF-32 Ti continuous flow rotor in a model L3–50 Spinco ultracentrifuge was used. The flow through the rotor was maintained by means of a Cole Parmer Masterflex model 7565 high capacity peristaltic pump with variable speed control. The temperature during all experiments was maintained below 4 °C.

Calf liver, obtained from a local slaughterhouse, was cut into pieces and thoroughly washed in a few volumes of

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