described above and found to be radioactive. It was supplied to strain W-1, and the red pigment consequently formed by the organisms was extracted by a modification of the method of WREDE AND HETTCHE[6] and was then chromatographed on paper. The paper was pretreated with propylene glycol, and the pigment was applied in toluene solution; chloroform was used as the mobile phase (*cf.* ZAFFARONI AND BURTON[7]). The pigment, which had the same R_F as authentic prodigiosin, was eluted with 50% aqueous methanol and rechromatographed on paper with hexane-glacial acetic acid (9:1). Upon elution, the red pigment showed the same absorption spectrum in the visible region as did authentic prodigiosin. These results indicate that the major pigment produced by strain W-1 in the presence of the isolated substance is indeed prodigiosin. Moreover, the prodigiosin thus formed was found to be labeled, its specific activity being equal, within experimental error, to that of the labeled material supplied to strain W-1. It is therefore concluded that the substance isolated from cultures of strain 9-3-3 is in fact a precursor of prodigiosin.

Strain 9-3-3 was generously supplied by Dr. M. I. BUNTING and strain W-1 by Dr. M. T. M. RIZKI. A sample of authentic prodigiosin was kindly provided by Mr. J. E. MCKEON. Valuable discussions with Drs. D. M. BONNER, M. I. BUNTING, and M. T. M. RIZKI are gratefully acknowledged.

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The adenosine deaminase of crustaceans*

DUCHATEAU, FLORKIN AND FRAPPEZ[1–4] have reported that invertebrates contain adenase but no adenosine deaminase and that vertebrates contain adenosine deaminase but no adenase. FLORKIN[4] has concluded "The appearance of an enzymatic system for the deamination of nucleosides is a biochemical characteristic of vertebrates in contrast to invertebrates". On the other hand, WAGNER AND MITCHELL[5] have reported adenosine deaminase in the larva of *Drosophila melanogaster* and LENNOX[6] has found adenosine deaminase and guanase in blowfly larva. The present report deals with the demonstration of adenosine deaminase in two crustaceans, a lobster (*Homarus americanus*) and a local fresh water crayfish (unidentified species). We have been unable to detect adenase in extracts of the hepatopancreases of either of these crustaceans. The lobster hepatopancreas is a convenient source of adenosine deaminase that is relatively free of other enzymes of purine metabolism.

The animals were killed by decapitation, the hepatopancreases removed immediately and homogenized with five volume of 0.1 ionic strength pH 7.0 phosphate buffer in a glass homogenizer. The extraction and all subsequent steps were carried out near 0° C. The homogenates were centrifuged for an hour at 20,000 × *g* and the clear supernatants were used in tests for the various enzyme activities. An extract of lobster hepatopancreas was fractionated with ammonium sulfate and most of the adenosine deaminase precipitated between 0.5 and 0.7 saturation. Acetone powders of the hepatopancreases were prepared; these and the fresh tissues were extracted with pH 9.5 borate buffer for 20 min at 40° C[7] to determine if uricase could be removed. Enzyme activities were measured spectrophotometrically with the following substrates and by methods indicated in the references: adenosine deaminase, adenine deoxyriboside deaminase, adenosine-3′-phosphate and adenosine-5′-phosphate deaminases, xanthine oxidase, nucleoside phosphorylase, and uricase[8]; adenase[9]; guanase, guanosine and guanine deoxyriboside deaminases[10]; cytosine deaminase[11]; purine-purine transribosidase and purine-purine transdeoxyribosidase[12].

The positive findings are listed in Table I. Adenosine deaminase and a very weak guanase activity were found in the crayfish hepatopancreas extracts; adenosine deaminase, guanase, and

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a weak nucleoside phosphorylase activity were found in the lobster hepatopancreas extracts. Reactions that were run in trishydroxymethylaminomethane buffer gave the same deamination rates for adenosine and adenine deoxyriboside as listed in Table I with phosphate buffer. The lobster hepatopancreas extracts gave negative results (no spectral change was noted) in the tests listed above and not included in Table I. The crayfish hepatopancreas extracts contained no adenase, inosine phosphorylase, adenosine-5'-phosphate deaminase, xanthine oxidase, or uricase.

TABLE I

ENZYMES OF PURINE METABOLISM FOUND IN CRUSTACEAN HEPATOPANCREAS EXTRACTS

Activities were measured at 25° C in 0.1 ionic strength pH 7.0 phosphate buffer.

Tissue	*Enzyme*	*Specific activity (μM/h/mg protein)*
Crayfish hepatopancreas	Adenosine deaminase	0.12
	Guanase	0.007
Lobster hepatopancreas	Adenosine deaminase	0.22
	Guanase	0.22
	Inosine phosphorylase	0.058
Lobster hepatopancreas, 0.5–0.7 sat. fraction	Adenosine deaminase	0.66
	Adenine deoxyriboside deaminase	0.28

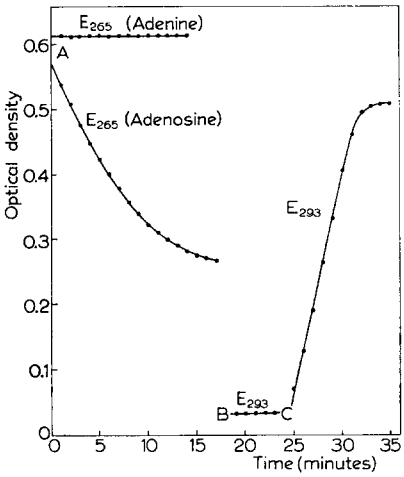

Fig. 1. At A, 0.1 ml of the purified lobster hepatopancreas adenosine deaminase was added to 3.0 ml of a 50 μM solution of each substrate (adenine and adenosine) in 0.1 ionic strength pH 7.0 phosphate buffer. At B, 0.03 ml of purified milk xanthine oxidase was added to the solution containing the adenosine reaction product and, at C, this was followed by the addition of 0.05 ml of purified beef liver nucleoside phosphorylase.

The presence and identity of adenosine deaminase in the lobster hepatopancreas and the absence of adenase is shown in Fig. 1. There is no change in optical density at 265 mμ when the extract is added to adenine; with adenosine, there is a rapid decrease in absorbancy at 265 mμ. That the product in the latter reaction is inosine and not hypoxanthine is shown by the addition of milk xanthine oxidase followed by beef liver nucleoside phosphorylase No reaction is noted with the xanthine oxidase until hypoxanthine is liberated by the action of nucleoside phosphorylase.

The finding of adenosine deaminase in various invertebrates ([5,6] and above work) and the failure to find adenase in the two crustaceans indicates that the hypothesis of FLORKIN[4] regarding the position of these enzymes in the evolution of animals must be revised. Perhaps the presence of adenosine deaminase and guanase in the crustaceans indicates a need for protection against the pharmacological action of the substrates adenosine and guanine[13].

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