were used in these studies. Samples of digestive gland tissue (hepato-pancreas) of 150-350 mg were homogenized in a buffered solution of sterile sea water. Rates of respiration, measured in μl O_2 consumed/ml homogenate/min, were determined for homogenates over a pH range of 5.2-8.1. It was found that peaks of maximum uptake .0023 μl O_2/ml/min ± .007 and .0025 μl O_2/ml/min ± .004 for Thais lapillus occurred at pH 5.4 and 6.6, respectively. Minimum values of about .0011 μl O_2/ml/min were recorded at pH 6.2 and 7.7.

Both species of pagurids showed maximum O_2 uptake at pH 7.7: for P. acadianus .0042 ± .0008 and for P. pubescens, .0050 ± .0002 μl O_2/ml/min. Lowest respiration rates .0029-.0030 were observed at pH values between 5.2 and 5.4. Because P. pubescens showed consistently higher rates than P. acadianus, protein analyses of homogenates were made using the Biuret technique. The results indicated that the two crabs did not differ regarding percentage protein by wet weight of hepato-pancreatic tissue. These ranged from 4.97% to 8.01%, and are close to the values cited by Watermann (The Physiology of Crustacea, 1960, vol. I, 302) for Cancer pagurus. Manometric studies of the metabolic rate of the crab, Pagurus hirsutiusculus (Life Sci. 1963, 131-33) show that 96% of the variation in oxygen consumption is explainable by variation in the weight of individuals used. In the present studies it seems reasonable to assume that differences in O_2 uptake rates in tissue homogenates of equal volume from the two crab species were due to endogenous factors unrelated to size.

Crabs of both species were placed in a heated, oxygenated aquarium at a temperature of 23° C ± 1.5. Others were placed in a cage sunk in Salisbury Cove (11° C - 12° C) at a depth of 30 feet for 72 hours. After 72 hours in the heated aquarium, the rates for P. acadianus were .0030 ± .0006 μl O_2/ml/min. All but one of the P. pubescens died. The respiration of tissue from both species from the sunken cage averaged .0030 μl O_2/ml/min. That rates of O_2 uptake varied so little between these two experimental groups, indicates that respiration of the hepato-pancreatic tissue is to at least some extent, independent of the past thermal history of individuals. This is significant, for it implies that the two pagurids possess mechanisms for regulating internal metabolic rates which are detectable in whole tissue homogenates.

Hepato-pancreas homogenates from recently caught crabs at pH 7.7 - 8.0 showed respiration rates of .0042 ± .0007 and .0051 ± .0002 μl O_2/ml/min for P. acadianus and P. pubescens respectively. The wide difference of respiration rates between these crabs and those caged for three days with little food, demonstrates the ability of specific tissues to compensate for environmental change which is detectable at the sub-cellular level. The detection of such compensation by oxygen monitoring of endogenous respiration in tissue homogenates may prove an effective method for determining general physiological response to environmental perturbation.

This work was supported by NSF Grant No. GY-7493 and Williams College research funds.

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EFFECTS OF CHLORAMPHENICOL AND CYCLOHEXIMIDE ON PROTEIN SYNTHESIS AND MORPHOGENESIS IN EMBRYOS OF Fundulus heteroclitus

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In order to investigate mechanisms of control over the events of early embryogenesis, we

have given much attention to the temporal relationship of protein synthesis to recognizable functional developments. For these studies it has been especially useful to control periods of protein synthesis in the intact embryo with inhibitors. Thus it is possible to relate the effect of a specific period of protein synthesis on the subsequent development of structures and functions. It has recently become apparent that inhibitors can also be used to distinguish between loci of protein synthesis. Cycloheximide has been shown to be an effective inhibitor of ribosomal protein synthesis while chloramphenicol inhibits synthesis in the mitochondria. Therefore it became of interest to determine whether these inhibitors in embryos of Fundulus heteroclitus could be of use to relate both time and locus of protein synthesis to future developmental patterns.

Eggs obtained from ripe females were fertilized by mixing with a sperm suspension obtained from minced testes. Incubations were carried out at 18° C. For determination of protein synthesis embryos of appropriate stage were selected from the culture and incubated for two hours with 3 μc ^{14}C-valine. The radioactive TCA-insoluble fraction was then isolated from the embryos and its specific activity determined. Gross analysis of morphogenesis was performed with the dissecting microscope and embryos for histological evaluation were appropriately prepared.

Cycloheximide showed very little tendency to inhibit valine incorporation into protein at the concentration levels usually employed for these studies (20 μg/ml). At 100 μg/ml the level of inhibition was approximately 40%. It does not appear to be a useful inhibitor of protein synthesis in the intact Fundulus embryo.

Chloramphenicol proved to be a potent inhibitor of valine incorporation into Fundulus protein. At inhibitor concentrations of 10 μg/ml the inhibition was over 95%. This proved to be true for all stages of development. However there is considerable doubt that these data indicate an inhibition of protein synthesis. Repeated studies on the morphogenetic behavior of chloramphenicol-treated embryos revealed little or no effect produced by the inhibitor. Length of exposure time of the embryos to chloramphenicol varied from 15 minutes to continuous and concentrations ranged from 20 μg/ml to 200 μg/ml. Only when the embryos were left in chloramphenicol continuously at 200 μg/ml did some minor disturbances in morphogenesis appear. A small percentage developed albino characteristics and died. The rest showed minor delays in development, recovered, and then hatched as normal fry. It is not conceivable that all this could happen in the absence of protein synthesis. Inhibitors of protein synthesis such as pactamycin produce regular patterns of developmental aberration, depending upon dose, pulse length, and pulse initiation time. Therefore it seems most reasonable to suspect that failure of valine incorporation into protein in the presence of chloramphenicol is the result of an effect upon permeability of the embryo to valine rather than upon an effect on the protein synthesis process.

Failure to incorporate an amino acid into protein is not sufficient evidence for inhibition of protein synthesis in intact systems. Certainly the developing embryo should show disturbances in its morphogenetic patterns if no proteins are being synthesized over a long period of time. Therefore, these studies demonstrated that in intact Fundulus embryos chloramphenicol and cycloheximide are not useful for determining the relative contributions of ribosomal and mitochondrial sites of protein synthesis.

These investigations were supported by NSF Grant GB-6766.