The Interaction of Fraction F2(b) from Calf Thymus Histone with Deoxyribonucleic Acid

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It has been found that the state of aggregation of histone fraction F2(b) in solution depends on the concentration of NaCl. It seemed likely that the interaction between this histone and DNA would be affected by changes in the degree of aggregation of the histone. As a preliminary to studies on the interaction with the homologous DNA the nature of the random reaction with a heterologous DNA has been studied. For these experiments solutions of the homogeneous DNA isolated from bacteriophage T7 have been used.

Equal volumes of DNA (120 $\mu\text{g./ml.}$) and histone (0–120 $\mu\text{g./ml.}$) were rapidly mixed and the amount of DNA left in solution was determined from experiments in the Spinco analytical ultracentrifuge at 42000 rev./min. with u.v.-absorption optics. From these experiments it was found that the amount of histone required to precipitate all DNA from solution depends on the NaCl concentration, e.g. histone/DNA ratio (w/w) for complete precipitation being 0.96 in 0.1M-NaCl and 1.64 in 0.005M-NaCl.

In contrast with the sharp boundary seen in sedimentation experiments with T7 DNA alone, the u.v.-absorbing material left in solution after mixing DNA and histone exhibits a broadening boundary typical of a heterogeneous solute. The mean sedimentation coefficient of the DNA-histone complex left in solution is higher than the sedimentation coefficient of an equivalent concentration of DNA alone, the difference in sedimentation rate increasing as the amount of complex left in solution decreases. The observations suggest that the particles of nucleoprotein complex formed under these conditions each contain more than one molecule of DNA.

Enzymic Phosphorylation of Histone F1 in Regenerating Rat Liver

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The occurrence of a specific protein kinase which catalyses the transfer of terminal phosphate of ATP to phosphoproteins in a variety of tissues has been known for some years (Burnett & Kennedy, 1954; Rabinowitz & Lipmann, 1960). Langan & Smith (1967) reported phosphorylation of histones and protamine by liver extracts. Ord & Stocken

(1966) and Stevely & Stocken (1968) have shown that the phosphate content of histones from livers of partially hepatectomized rats, which show increased DNA synthesis, is greater than that of control animals. A detailed study of histone kinases was therefore initiated to assess whether the enzymic activity could also be correlated with events in the mitotic cycle.

The soluble supernatant (20000g) of rat liver homogenate was the source of the enzyme, with calf thymus histone F1 as the recipient and [γ - ^{32}P]-ATP as the phosphate donor. Studies at different time-intervals after hepatectomy showed a rise in the activity of the enzyme at 16 hr. of regeneration, with a peak at 22 hr., paralleling the increased phosphate content of histone F1 found in this time-period. Although the phosphate content of histone F1 varies through the day (Ord & Stocken, 1968), indications of such diurnal variations were not present with the histone kinase enzyme system.

Radiosensitive phosphorylation of histone F1 has been observed (Ord & Stocken, 1968) in the livers of partially hepatectomized rats, and the effect of ionizing radiation on the kinase was therefore examined. If the animals were irradiated (500 rads) 3 hr. after the operation and killed at 22 hr. there was a 60–70% decrease in the activity of the enzyme compared with control rats that had not been irradiated. When the rats were exposed to ionizing radiation during the period of DNA synthesis there was a 20–25% decrease in the specific activity. The enzyme in livers from intact adults was practically unaffected by γ -irradiation. Partially purified preparations exposed to 10000 rads *in vitro* also did not show any significant inactivation.

These results indicate that histone F1 kinase in regenerating liver behaves in a manner similar to thymidylate kinase in response to the stimulus of hepatectomy and irradiation.

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