of genetic studies in inbred mice and submolecular characterization of the immunoglobulins should lead to further insight into the genetic-structural relationship of these important proteins.

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6 January 1964

Polymer Similar to Polydeoxyadenylate-Thymidylate in Various Tissues of a Marine Crab

Abstract. *Several species of a genus* Cancer *have a DNA that has a light buoyant density and that contains mainly deoxyadenylate and thymidylate. The presence of this polymer was demonstrated previously in testes and vas deferens. By a modified procedure for isolating DNA, the muscles, liver, and eggs of* Cancer borealis *are also shown to contain the deoxyadenylate-thymidylate-like polymer.*

Certain marine crabs both in the Atlantic Ocean (*Cancer borealis* and *Cancer irroratus*) (*1–3*) and in the Pacific Ocean (*Cancer antnnearius, Cancer gracilis,* and *Cancer magister*) (*4*) have a DNA component whose base composition is mostly deoxyadenylate-thymidylate. In the case of *C. borealis,* this polymer consists of 30 percent of the total DNA of the testes. The polymer was isolated free from the main DNA and was found to have a double helical structure and density and thermal hyperchromic shift similar to those of deoxyadenylate-thymidylate polymer which had been enzymatically synthesized (*2, 3*). The polymer acts as a primer for DNA polymerase, and the deoxyadenylate and deoxythymidylate residues are in alternate sequence (*5*). It contains 2.7 percent guanine and cytosine, and there is a tendency for deoxyguanylic residue to replace deoxyadenylic rather than thymidylic and for the deoxycytidylic residue to replace the thymidylic (*5*). The polymer also acts as an excellent primer for RNA polymerase isolated from *Micrococcus lysodeikticus* (*6*).

The existence of the extra DNA component in different tissues of the crab was not clear because of the difficulty of obtaining highly polymerized DNA from tissues other than testes and vas deferens. With a different method of DNA isolation, we have been able to characterize the DNA of the muscle, liver, and eggs of *C. borealis.* Centrifugation of the material in a CsCl gradient shows that the DNA from the muscle, liver, and eggs contains the polymer in approximately the same amount, and density identical to that of the light DNA component in the testes and vas deferens.

Live crabs were dissected, and the testes, claw muscle, liver, and eggs were washed separately with an EDTA-saline solution (0.1*M* ethylenediamine tetraacetate, 0.15*M* NaCl, *p*H 8.0). The testes were frozen immediately and kept in the freezer. The washed muscle, liver, and eggs were quickly frozen by dipping them into liquid nitrogen. The tissues were kept in liquid nitrogen until they were used for DNA isolation.

Table 1. Relative amounts of the main DNA and "dAT" in different tissues of *Cancer borealis.**

Tissues	Main DNA (%)	"dAT" (%)
Testes	69	31
Liver	68	32
Muscle (claws)	68	32
Eggs	66	34

* Calculated from tracings of ultraviolet absorption pictures of equilibrated DNA in the CsCl density gradient.

The procedure for isolating DNA from crab testes has been described (*2*). Approximately 20 g of muscle was ground in a chilled mortar with an occasional addition of liquid nitrogen. The frozen powder was mixed with 70 ml of cold EDTA-saline plus duponol (2.5 percent) in a teflon-pestle tissue grinder, while the temperature was kept below 4°C. Fourteen milliliters of 5*M* sodium perchlorate were added to the mixture. The suspension was shaken with a mixture of isoamyl alcohol and chloroform (4:96 by volume) at room temperature for 60 minutes. The mixture was centrifuged at 4°C at 5000*g* for 15 minutes. The top aqueous layer was shaken with a mixture of isoamyl alcohol, chloroform, and phenol (3:72:25) for 30 minutes and the mixture was centrifuged. The resulting top layer was then shaken with a mixture of isoamyl alcohol, chloroform, and phenol (2:48:50) and centrifuged. To the final aqueous portion, two volumes of 95 percent ethanol were added to precipitate nucleic acids. The precipitates were collected by centrifugation and dissolved in 10 ml of standard saline citrate (0.15*M* NaCl plus 0.015*M* sodium citrate) to which a preheated ribonuclease solution (*7*) was added, making the ribonuclease concentration 50 μg/ml. After incubating the mixture at 37°C for 30 minutes, one-fourth volume of 5*M* sodium perchlorate was added and shaken with an equal volume of a mixture of isoamyl alcohol, chloroform, and phenol (2:48:50) at room temperature for 15 minutes. Macromolecules were precipitated with ethanol as described, and the precipitates were dissolved in 5 ml of standard saline citrate. The solution had a total of 300 units of absorbance at 260 mμ. A portion of the solution (1 ml) was diluted with saline citrate to an absorbance at 260 mμ of 0.8 and applied to a one-layer methylated albumin-kieselguhr (MAK) column (*2*). The column was prepared with 10 ml of a suspension of MAK (*2*) and had a packed size of 1.5 cm in diameter and 1 cm in height. The charged

column was washed by passing through 25 ml of 0.4*M* saline phosphate buffer (0.4*M* NaCl, 0.05*M* sodium phosphate buffer, *p*H 6.7) and then through 25 ml of 0.55*M* saline phosphate buffer. The DNA was eluted in steps with 2-ml portions of 1.1*M* saline phosphate buffer. A large amount of the ultraviolet absorbing materials (unidentified) came out in a 0.4*M* fraction. The fraction with the highest absorbance (at 260 mμ) eluted with 1.1*M* saline buffer was used for density gradient centrifugation.

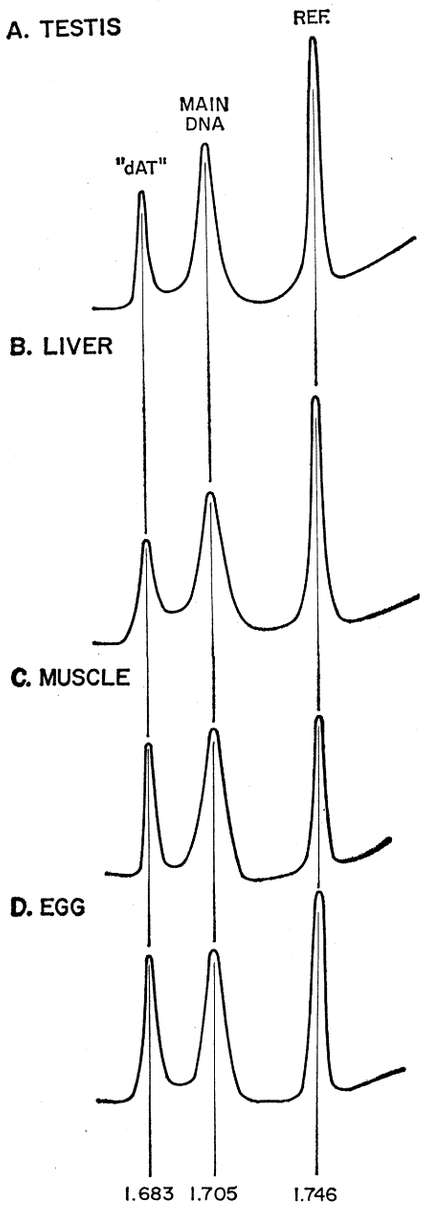

Fig. 1. Distribution of *Cancer borealis* DNA from various tissues in CsCl buoyant-density gradient. DNA samples (2 to 3 μg) were centrifuged with 1 μg N^{15}-*Pseudomonas aeruginosa* DNA as a reference at 44,770 rev/min in 7.7 molal CsCl solution at 25°C for 20 hours. Ultraviolet absorption photographs of equilibrated DNA were traced by a microdensitometer. Densities of the peaks were calculated (*1*). The density values were calculated relative to the density of *Escherichia coli* B DNA (1.713). *A*, DNA from testes; *B*, DNA from liver; *C*, DNA from claw muscle; *D*, DNA from eggs. The horizontal axis indicates buoyant density.

The isolation procedure for liver DNA was somewhat different from the method described for muscle. The frozen liver (5 g) was dipped in heated EDTA-saline (50 ml) and kept at 70°C for 15 minutes. After heating, the fragments of liver were collected by centrifugation, frozen in liquid nitrogen, and then ground in a mortar with a continuous supply of liquid nitrogen. The frozen powder was resuspended in 20 ml of the EDTA-saline buffer and 2 ml of duponol solution (25 percent), and ground in a teflon-pestle tissue grinder. The homogenate was treated with the mixture of isoamyl alcohol, chloroform, and phenol and with ribonuclease in the manner described for muscle. Apparently, a large amount of material other than DNA was precipitated by ethanol which could not be separated from the DNA by shaking with the organic solvents. The precipitates were dissolved in 3 ml of standard saline citrate, and a portion (1 ml) equivalent to 80 absorbance units at 260 mμ was diluted to 20 ml with standard saline citrate and applied to a 10-ml MAK column. The DNA was eluted as before. The column fractionation removed nonnucleic acid material and about 270 μg of DNA was obtained. The method used for isolation of DNA from eggs is similar to the method described for muscle DNA. From 15 g of eggs, approximately 1 mg of DNA was obtained.

The DNA samples from testes, muscle, liver, and eggs of *Cancer borealis* were centrifuged in 7.7 molal CsCl solution (*8*) at 25°C, 44,770 rev/min for 20 hours. The tracings of the ultraviolet absorption pictures are shown in Fig. 1, and relative amounts of the main DNA and light DNA ("dAT") in Table 1. The results show that muscle, liver, and egg samples as well as that of testes have a light DNA band whose density is identical with the light DNA previously described in testes.

In conclusion, the natural "dAT" first found in the testes and vas deferens of *Cancer borealis* exists in muscle, liver, and eggs as well, in approximately the same relative amount (30 percent). Consequently, it exists also in both male and female.

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Homocystinuria: An Enzymatic Defect

Abstract. *A deficiency, or absence, of cystathionine synthetase activity has been demonstrated in liver obtained from a mentally retarded child with homocystinuria.*

Homocystinuria was added recently to the list of biochemical abnormalities known to be associated with mental deficiency in man (*1*, *2*). Carson *et al.* (*2*) studied two homocystinuric sisters

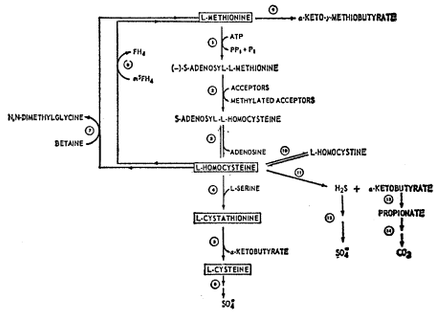

Fig. 1. Known pathways of mammalian metabolism of methionine and homocysteine. Abbreviations: ATP, adenosine triphosphate; PP_i, inorganic pyrophosphate; P_i, inorganic phosphate; FH_4, tetrahydrofolic acid; $^5FH_4$, N^5-methyltetrahydrofolic acid.