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A Defect in the 40s Ribosomal Subunit after Hypophysectomy of the Rat

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Hypophysectomy of the rat diminishes, and growth-hormone injection stimulates, the ability of isolated liver ribosomes to incorporate amino acids into protein in the absence or presence of excess of polyuridylic acid (Korner, 1968). We report the results of experiments designed to detect which of the ribosomal subunits has been effected by hypophysectomy.

Rat liver ribosomes were prepared by a method involving precipitation with Mg^{2+} ions (Takanami, 1960) to remove ferritin, which obscures the large ribosomal subunit on sucrose density gradients. The ribosomes were dissociated to subunits by adjusting the K^+ ion concentration of the medium to 0.8M and warming to 30° for 5 min. (Martin & Wool, 1968). The dissociated ribosomes were layered on 7–30% linear sucrose gradients containing 0.8M-KCl. After centrifugation at 2° in an MSE 3 × 20ml. rotor for 5 hr. at 100000g, the gradients were analysed at 260nm. The fractions corresponding to subunits were collected. Hybrid ribosomes were formed from some of them by mixing the 60s and the 40s subunits in the E_{260} ratio 2.5:1. The mixture was dialysed overnight to remove excess of KCl and sucrose, and the hybrids and the subunits were assayed for their ability to catalyse polyphenylalanine synthesis in the presence of polyuridylic acid. The conditions of incubation were as described by Munro, Jackson & Korner (1964).

The reconstituted ribosome made from the 60s and 40s subunits of normal liver ribosomes, and the hybrid made from a 40s normal subunit with a 60s subunit from hypophysectomized rat liver showed about equal activity. Any hybrid containing the 40s subunit from hypophysectomized rat, however, had less ability to incorporate [^{14}C]-phenylalanine into acid-precipitable material than hybrids containing the normal 40s subunit.

Martin & Wool (1968) found that ribosomes from muscle of diabetic rats, which are less active than normal ones, have a defect in the 60s subunit. Our results show that the defect resulting from hypophysectomy is in the 40s subunit.

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Conversion of 4-Thiouracil in Amino Acid-Transfer Ribonucleic Acid into N^4 -Methylcytidine for Sequence Analysis

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Although sequence analysis of oligonucleotides containing 4-thiouracil is hampered by the chemical reactivity of the sulphur atom, this feature can be used to convert 4-thiouracil into a stable pyrimidine which can, if desired, be selectively labelled with ^{14}C . OsO_4 is known to oxidize 4-thiouridine beyond the disulphide and the oxidation product(s) react readily with ammonia to form cytidine (Burton, 1967). With methylamine, the principal final product is N^4 -methylcytidine and the reaction can be applied to introduce [^{14}C]methylamino groups into amino acid-transfer RNA (t-RNA*). Analogous reactions of periodate-oxidized 4-thiouridine have been discovered independently by Ziff & Fresco (1968).

Typical conditions are to dissolve 1mg. of t-RNA in 70 μ l. of a solution containing 20mM- ^{14}C -methylamine (specific radioactivity 10c/mole) and 16mM-HCl. OsO_4 (10 μ l. of an aq. 90mM solution) is then added and the mixture is incubated for 1 hr. at 37°. Residual osmium compounds are reduced by incubation with 10 μ l. of mercaptoethanol for 10 min. The RNA is precipitated by ethanol and freeze-dried. Unfractionated t-RNA from *Escherichia coli* gives several radioactive oligonucleotides after digestion with ribonucleases and separation by the two-dimensional systems of Sanger, Brownlee & Barrell (1965). On complete degradation to nucleotides, N^4 -methylcytosine nucleotide was the only radioactive compound detected. The pancreatic nuclease digests support the conclusion of Lipsett (1968) in indicating that most of the 4-thiouridine is linked through its 5'-hydroxyl group to a purine nucleotide; nevertheless, Py-A-G-thioU and Py-G-A-thioU are rare sequences. In T-1 ribonuclease digests of the modified RNA, labelled nucleotides resembling (A, N^4 -methylC)-G are the most common. Purified preparations of phenylalanine t-RNA gave single radioactive spots in both T-1 and pancreatic-ribonuclease digests, and their behaviour on partial

* Abbreviations: t-RNA, transfer RNA; Py, pyrimidine; thioU, 4-thiouridine; N^4 -methylC, N^4 -methylcytidine.