GOLDFARB *et al.*[2] have recently interpreted the experiments of SHORE AND PARDEE[6] as evidence for energy transfer in proteins by means of an excited-peptide-bond mechanism, similar to conductance-band transfer. These authors have failed to recognize that the findings of Shore and Pardee are reaily interpreted by the resonance mechanism as is shown in this paper. Their most significant error in assuming that an excited-peptide-bond transfer mechanism can account for the energy transfer observed in the protein conjugates is that the peptide bond requires much higher energies of excitation than is provided in a quantum absorbed at 280 mμ by an aromatic amino acid[10].

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Zone-electrophoretic studies on soluble RNA from rat-liver cytoplasm

The soluble polynucleotides present in rat-liver cytoplasm, until recently almost unexplored, aroused the interest of many investigators in the field of protein biosynthesis for several reasons. Following activation of amino acids by ATP and specific enzymes, the amino acids are transferred to soluble RNA of low molecular weight. Incubation of this sRNA-amino acid complex with liver microsomes in the presence of GTP results in the incorporation of amino acid into microsomal ribonucleoprotein[1]. Isotope experiments *in vivo* revealed that sRNA becomes more highly labelled than microsomal RNA[2-4], while evidence has been presented for a metabolic transfer *in vitro* of the sRNA to the microsomal RNA [5]. Finally it was shown that CTP, ATP

Abbreviations: ATP, adenosine triphosphate; GTP, guanosine triphosphate; CTP, cytosine triphosphate; UTP, uridine triphosphate; RNA, ribonucleic acid; sRNA, soluble RNA.

and to a lesser extent UTP may serve *in vitro* as proximal precursors for the addition of mononucleotide units to the terminal nucleoside of sRNA[6].

In order to characterize this soluble RNA more thoroughly, pH 5 enzymes isolated from rat-liver cytoplasm were incubated with DL-[^{14}C]leucine and ATP according to the method of HOAGLAND *et al.*[1] and submitted to zone electrophoresis on starch[7]. After the electrophoretic run the starch block was cut into segments of 1 cm width. The segments were eluted with dilute phosphate, pH 7.4, and the eluates were assayed for radioactivity, RNA, and protein content. The results are presented in Fig. 1. It shows that labelled pH 5 enzyme yielded two zones representing protein and RNA respectively, 90–100 % of the radioactivity being in the RNA fraction. Free leucine does not migrate at pH 7.4 under our experimental conditions. In this paper we will only refer to radioactivity not residing at the origin, *i.e.* "bound" [^{14}C]leucine.

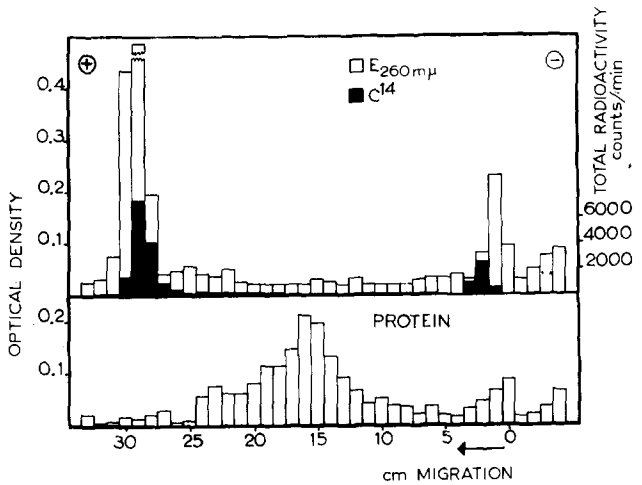

Fig. 1. pH 5 enzymes, containing about 25 mg protein and 1 mg RNA, were labelled with [^{14}C]-leucine according to the method of HOAGLAND *et al.*[1] and precipitated at pH 5.2. The precipitate was redissolved at pH 7.4 and submitted to starch-block electrophoresis which was carried out for 15 h at 4.5 V/cm (10–11 mA) in phosphate (I 0.025, pH 7.4). The starch block was kept in a vertical position so that electroosmotic flow was counterbalanced by gravitational forces[7]. 1-cm segments were extracted with dilute phosphate, pH 7.4. Protein content was estimated according to LOWRY *et al.*[8]. RNA was determined after hydrolysis of the eluates with 5 % $HClO_4$ by measuring the absorbance at 260 mμ. Aliquots of the eluates were plated directly, assayed for radioactivity and corrected for self absorption. Precipitation after incubation of the enzymes reduces the amount of free leucine at the origin.

It is essential that the starch block is kept at 1–3° during the electrophoretic experiment. The temperature was checked regularly by measuring the electrical resistance of a thermistor, which was inserted into the starch block. The temperature was computed from a calibration curve, relating temperature and electrical resistance. When the temperature rises above 5°, [^{14}C]leucine-RNA may dissociate to a relatively high extent into its components as becomes evident from the distribution of radioactivity along the starch block. The RNA labelled with [^{14}C]leucine appears as a rather sharp and distinct zone comprising 3 to 4 segments. That this RNA fraction is not homogeneous, however, may be concluded from the difference in specific activity of the RNA present in the segments 27–30 cm from the origin.

Several experiments similar to that described above did not reveal the presence of ribonucleoprotein or of free RNA of higher or lower mobility. HOAGLAND *et al.*[1] reported that the pH 5 enzyme represents only 20% of RNA of the supernatant fraction, obtained after centrifugation at 105,000 × *g* for 2 h.

In subsequent experiments we incubated the 105,000 × *g* supernatant with DL-[^{14}C]leucine and ATP for 10 min at 37°. One quarter of the incubation mixture was submitted to zone electrophoresis, the remainder was precipitated at pH 5.2. The precipitate was redissolved in one third of the original volume at pH 7.4 and the labelled pH 5 enzyme thus obtained was fractionated electrophoretically in the same run. (Two equal starch blocks were connected to the same electrode vessels.)

Fig. 2 shows the results of the electrophoresis experiment with the incubation mixture containing the 105,000 × *g* supernatant. In this case no separation between

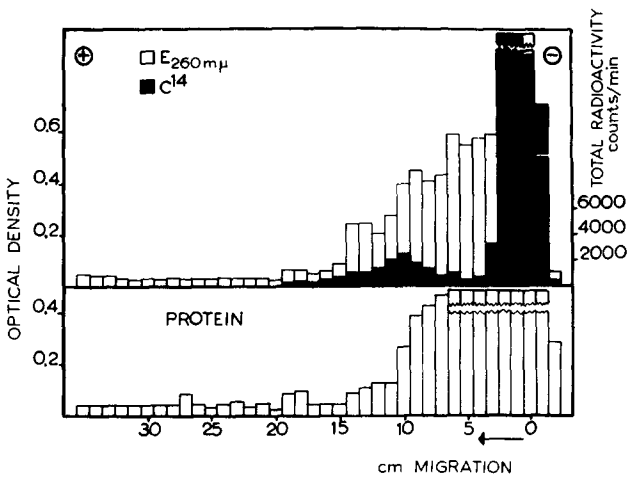

Fig. 2. Starch-block electrophoresis of an incubation mixture containing 4.5 ml 105,000 × *g* liver supernatant, 0.8 μC DL-[^{14}C]leucine and 2.5 μmoles ATP (total vol. 5.0 ml), incubated at 37° for 10 min. For experimental conditions of the electrophoresis, see Fig. 1.

RNA and protein was obtained. Moreover, radioactivity did not move as far as in Fig. 1. It was found in segments containing both protein and RNA and hence might be linked to ribonucleoprotein. Following precipitation of the incubation mixture at pH 5.2, electrophoretic fractionation (at pH 7.4) yielded a sharp RNA peak in the same position as the RNA in Fig. 1, containing [^{14}C]leucine. It is assumed that due to the lowering of the pH to 5.2, [^{14}C]leucine–RNA dissociates from protein and migrates faster towards the anode than does the intact ribonucleoprotein. In contrast to Fig. 1 radioactivity in Fig. 2 appeared in a rather wide zone comprising about 10–15 segments. This zone probably represents a mixture of labelled compounds, one of which is ribonucleoprotein linked to [^{14}C]leucine. When the incubation mixture containing 105,000 × *g* supernatant, [^{14}C]leucine and ATP was extracted with phenol[9] about half of the "bound" [^{14}C]leucine during electrophoresis moved to positions intermediary between the labelled RNA of the pH 5 enzyme and the origin.

Fig. 3 illustrates the results obtained by starch-block electrophoresis of such a phenol extract (the extract was freed of phenol by ether extraction and lyophilyzed before electrophoresis). At least two peaks absorbing at 260 mμ emerged, one corre-

sponding to [^{14}C]leucyl-RNA precipitable at pH 5.2 and containing about 40 % of the radioactivity. 60 % of the radioactivity, however, resided in the segments 12–20 cm from the origin. One might expect this radioactive zone to contain [^{14}C]leucyl-AMP. The distribution of radioactivity over so many segments, however, suggests that it is heterogeneous and it is tempting to believe that it represents [^{14}C]leucine linked to smaller polynucleotides. The elucidation of the nature of this radioactive material has to await further experimentation.

The starch-block-electrophoresis experiments reported here suggest that, in the native state, soluble RNA occurs as ribonucleoprotein which precipitates and dissociates from protein at pH 5.2. This RNA is not homogeneous and only part of it may be considered as the polynucleotide carrier of L-leucine (see Fig. 1) although other carriers may not be excluded (see Fig. 3). The so called pH 5 enzymes isolated from rat liver do not contain ribonucleoprotein.

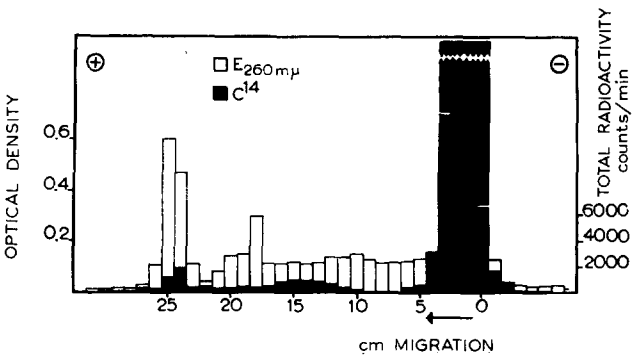

Fig. 3. Starch-block electrophoresis of a phenol extract obtained by extracting the following incubation mixture: 10 ml 105,000 × *g* liver supernatant, 7,5 μmoles ATP, 12 μC DL-[^{14}C]leucine (total vol. 15 ml), incubated at 37° for 10 min. For the experimental conditions of the electrophoresis see Fig. 1.

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