cellular sites. The difference in the pH optima of the mevalonate-kinase isoenzymes appears to be far greater than is usually found between isoenzymes. Though L-aspartate-2-oxoglutarate aminotransferases of mitochondria and the 100 000g supernatant of rat-liver homogenates have optima over the range pH 5.5–9.0 and at pH 8.0–9.0 respectively, the latter enzyme still shows appreciable activity (30%) at pH 5.5 (Fleischer, Potter & Wakim, 1960; Boyd, 1961). A number of acid phosphatases exist in different mammalian tissues that show pH optima varying from pH 4.0 to pH 6.0 (Moore & Angeletti, 1961). Commonly, however, pH optima of isoenzymes differ very little. Thus the three arylesterase isoenzymes in serum have optima at pH 7.9, 7.9 and 8.5 respectively (Wilde & Kekwick, 1964). For the β -glucuronidases of mitochondria (optimum pH 4.7) and microsomes (optimum pH 5.2) of rat liver the difference in optima can be attributed to combination of the microsomal enzyme with ribonucleic acids (de Duve, Pressman, Gianetto, Wattiaux & Appelmans, 1955).

Differences in intracellular localization of isoenzymes in higher plants have been reported but usually, as with the malate dehydrogenases of mitochondria and cell sap of pea epicotyls, the isoenzymes show almost identical properties (Davies, 1961).

The existence of mevalonate kinases inside and outside the chloroplast supports the view that there are two distinct pathways of terpenoid biosynthesis in plant cells and suggests that other enzymes in chloroplasts may show similar differences compared with enzymes catalysing the same reactions else-

where in the cell. Interpretations of results obtained with homogenates of plant cells should take into consideration the possibility of such differences, and the extrapolation of results from such preparations to situations within intracellular organelles should be made with caution.

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Electrophoretic Separation of Cytoplasmic Ribonucleic Acid Fractions of Pancreas and Liver

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Electrophoretic separation of different types of RNA has been achieved by using agar or starch gels as supporting media. Bachvaroff & McMaster (1964) resolved the RNA of microsomes of liver and of lymph nodes into five electrophoretic bands by agar-gel electrophoresis. Tsanev (1965) used a similar method to separate the RNA fractions of

rat liver. Székely, Beney, Vincze, Gaál & Tamás (1966) separated the ribosomal RNA of pigeon pancreas into four main fractions and some minor components by starch-gel electrophoresis. The electrophoretic patterns of RNA prepared from different cytoplasmic fractions of liver and pancreas were compared in the present experiments and the

electrophoretic bands were identified with RNA fractions of known sedimentation characteristics. A simple technique for the separation of t-RNA* and t-like RNA is also described.

Methods. Pigeon liver or pancreas was homogenized in 5 vol. of 0.25M-sucrose solution, pH 7.4, containing bentonite (0.1 mg./ml.). The total cytoplasmic fraction was obtained after removing cell debris and nuclei by centrifugation at 1000g for 10 min. After centrifugation at 10000g, the resulting supernatant was treated with 0.5% sodium deoxycholate in the presence of MgCl₂ (0.1–1.0 mM), and the ribosomes were sedimented at 105000g in 2 hr. RNA was isolated from the total cytoplasmic fraction, from the ribosomes or from the 105000g supernatant, after treatment with sodium dodecyl sulphate (Kurland, 1960), by the phenol method of Kirby (1956). RNA was precipitated with ethanol. The bulk of glycogen was removed from the preparations by dissolving the precipitate in a small volume of ice-cold water and centrifuging off the insoluble material at 0°.

* Abbreviations: t-RNA, transfer RNA; t-like RNA, transfer-like RNA.

Starch gels were prepared as described by Székely *et al.* (1966). Electrophoresis was performed in 16% starch gels containing phosphate buffer, pH 6 (10 mM), and urea (4 M), at 4 v/cm. for 210 min. RNA bands were detected by staining with Toluidine Blue.

Results. Four main bands (A, B, C and D in Fig. 1a) were present in the electrophoretograms of RNA preparations obtained from the whole cytoplasmic fraction or from the ribosomes of liver. These two RNA patterns differed merely in that band D was much stronger in the preparations of total cytoplasmic RNA containing also the soluble RNA fraction (Figs. 1a and 1b). Band D was identified with soluble RNA in electrophoretograms of RNA isolated from the 105000g supernatant (Fig. 1c). The RNA pattern of the cytoplasm of pancreas was essentially identical with that of liver (Fig. 1g). Pancreatic ribosomes contained in addition some components appearing in the electrophoretograms as weaker bands between bands B and C and beyond band D.

The main electrophoretic bands were identified by subjecting to starch-gel electrophoresis the

Fig. 1. Electrophoretograms of RNA in 16% starch gel containing phosphate buffer, pH 6 (10 mM), and urea (4 M). The voltage gradient was 4 v/cm. and the duration of electrophoresis was 210 min. (a) RNA of liver whole cytoplasm; (b) RNA of liver ribosomes; (c) RNA of liver 105000g supernatant; (d) RNA of pancreas ribosomes; (e) and (f) t-like RNA and t-RNA respectively separated by molecular sieving of the ribosomal RNA preparation giving electrophoretogram (d); (g) RNA of pancreas whole cytoplasm; (h), (i) and (j) 28s, 18s and 4s RNA respectively separated by gradient centrifugation of an RNA preparation from pancreas whole cytoplasm; (k) and (l) t-like RNA and t-RNA respectively separated by molecular sieving of the cytoplasmic RNA preparation giving electrophoretogram (g).

pooled 28s, 18s and 4s RNA fractions obtained by centrifugation of the total cytoplasmic RNA preparations in a sucrose density gradient. As shown in Figs. 1(h), 1(i) and 1(j), band *A* corresponds to 28s RNA and band *B* to 18s RNA, whereas the 4s RNA fraction obtained by density-gradient centrifugation was resolved into two bands, *C* and *D*, by gel electrophoresis. The appearance of two bands could not be ascribed to separation of free t-RNA and of aminoacyl-t-RNA, as after mild alkaline hydrolysis (pH 9) of the RNA preparation neither band *C* nor *D* disappeared from the electrophoretic patterns. The patterns shown in Figs. 1(b) and 1(c) clearly show that only band *D* corresponds to soluble RNA and that band *C* is a separate RNA fraction characteristic of the ribosomes.

The presence of a t-like RNA in bacterial as well as in animal cells has been described (Rosset & Monier, 1963; Virmaux, Mandel & Urban, 1964; Galibert, Larsen, Lelong & Boiron, 1965; Comb, Sarkar, De Vallet & Pinzino, 1965; Schleich & Goldstein, 1966). This RNA, characterized by nucleotide composition, molecular weight and sedimentation coefficient similar to but not identical with that of true t-RNA, is associated with ribosomes. Its possible identity with the ribosomal RNA fraction giving rise to the electrophoretic band *C* was therefore checked. Working with ribosomal RNA of liver and of KB cells, Galibert *et al.* (1965) separated t-like RNA of sedimentation coefficient 5s from t-RNA of sedimentation coefficient 4s by molecular sieving on a Sephadex G-100 column. By applying their technique to cytoplasmic RNA of liver and of pancreas we isolated the t-RNA and t-like RNA fractions and applied them to starch gel for electrophoretic identification. In the electrophoretograms t-like RNA gave a single band with the electrophoretic mobility of band *C*, whereas t-RNA could be identified with the component appearing in the electrophoretograms as band *D* (Figs. 1k and 1l). The same results were obtained when t-like RNA and t-RNA isolated by gel filtration of ribosomal RNA were subjected to gel electrophoresis (Figs. 1e and 1f). (As molecular sieving does not separate 5s RNA from some degradation products of ribosomal RNA, in a few cases the t-like RNA fraction was contaminated by such fragments and was resolved into more bands on electrophoresis.)

No 5s RNA band was detected in the 105000g supernatant.

The additional bands in the pattern of ribosomal RNA from pancreas (Fig. 1d) represent degraded RNA fractions. Studies on the stability of ribosomes (L. Beney & M. Székely, unpublished work) indicate that high-molecular-weight RNA is broken down in pancreatic ribosomes during isolation of the particles. The electrophoretic mobilities of the components moving faster than 4s RNA agree with those of RNA fragments obtained by treatment with ribonuclease (Székely *et al.* 1966). These bands can thus be considered as low-molecular-weight degradation products. If present, they were found in the 4s RNA fraction obtained by either gradient centrifugation or molecular sieving. By the same methods it was shown that the weak bands between *B* and *C* represent higher-molecular-weight breakdown products of RNA. They appeared in the patterns of RNA preparations, sucrose-density-gradient analysis of which showed signs of denaturation and degradation.

Better separation of different native RNA as well as degraded RNA was achieved by starch-gel electrophoresis than by the methods of gradient centrifugation or molecular sieving. This good resolution made it possible to use the electrophoretic technique also as a tool for checking 'nativeness' and for following changes of the macromolecular structure of ribosomes.

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