the two main bands (*a* and *b*) which had a lower protein concentration than commonly observed in normal homozygous individuals, a more slowly moving band was seen. If two molecular species of α_1-at are present in these individuals, and both species can be resolved into three components, the observed pattern could be explained by partial overlapping of the bands. The slower of the two main bands of the normal α_1-at would coincide with the faster main band of the variant, thus leading to the higher protein concentration of the middle band (*a*) as seen in Fig. 1. This interpretation is consistent with the antigen-antibody crossed-electrophoresis experiments, in which the height of the peaks is a function of the antigen concentration (Fig. 2B). The weak anodal band (*c*) is unlikely to be clearly visible because, in the heterozygous state, the concentration of the protein should be approximately 50 percent of that normally present in this position. With the more sensitive antigen-antibody crossed electrophoresis, however, a small amount of material can be detected in position *c*.

The third phenotype examined was from an individual heterozygous for the α_1-at deficiency gene in whose serum half the normal trypsin inhibitory capacity was found. In this case, a normal but more weakly staining banding pattern was observed (Fig. 1, +/−). The observed phenotypic variations in the serum α_1-at appear similar to those electrophoretic patterns reported under the designation of "prealbumin" variants (*8*). Since the *p*H of the buffer in our experiments was similar to that used for the disclosure of the "prealbumin" variants, these bands probably represent variations in the serum α_1-at. If this is correct, the phenotypes not yet seen are those designated "SS" and "FS" (*8*). The "SS" pattern had one strong band approximately in position *a*, a second strong band moving cathodally of *a*, and a faint one in position *b*. The "FS" pattern showed three weak bands in positions *a*, *b*, *c*, and one moving cathodally of *a* (Fig. 1). The pattern of the "SS" phenotype would be expected to occur in an individual homozygous for the slow variant, whereas the "FS" pattern would occur in an individual who was heterozygous for the α_1-at deficiency and the slow variant. Such an observation would imply that the deficiency gene and the gene for the slow variant are probably situated at two different loci. Several authors have observed genetic variation in the electrophoretic region preceding the albumin band in species other than man (*9*). Some of these genetic variations may also represent variations in the serum α_1-at.

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10. Some of the serum samples used in the experiments were kindly supplied by Dr. C. Hames, Claxton, Georgia. Work supported in part by PHS grant GM-577 from the Institute of General Medical Sciences and by PHS grant AM01542-10 and PHS grant HEO-3341-09.

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Ribosomes from Pear Fruit

Abstract. *Ribosomes, subunits and polysomes, have been isolated from cells of ripening (senescent) fruit. Sedimentation in sucrose gradients, sedimentation constants, and electron micrographs confirm the physical resemblance of fruit ribosomes to those from other living tissues.*

Ribosomes have been isolated from pea seedlings (*1*), corn root tips (*2*), and several other actively growing plant tissues (*3*), but there are no reports of their isolation from the moribund cells of senescent (ripening) fruit. Fruit tissues have been used extensively in the study of cellular senescence (*4*) and, more recently, in that of intracellular responses to massive irradiation (*5*). Since both phenomena are underlain by protein synthesis and associated mechanisms, the isolation of ribosomes is vital to their further study.

Paucity of cytoplasm and interference from vacuolar contents, principally acids and phenolics, impede the isolation of intracellular organelles from fruit tissues. To increase cell breakage and effect good control of *p*H, we immersed 120 g of peeled and grated fleshy tissue from D'Anjou pears in liquid nitrogen in a large mortar along with 90 ml of medium containing 0.01 *M* potassium phosphate buffer, *p*H 6.4; 240 μmole of cysteine; 5 percent sucrose; 6 m*M* of $MgCl_2$ plus 12 ml of 1 percent deoxycholate and 12 ml of 10 percent polyvinylpyrrolidone (PVP); and sufficient 1 *M* KOH

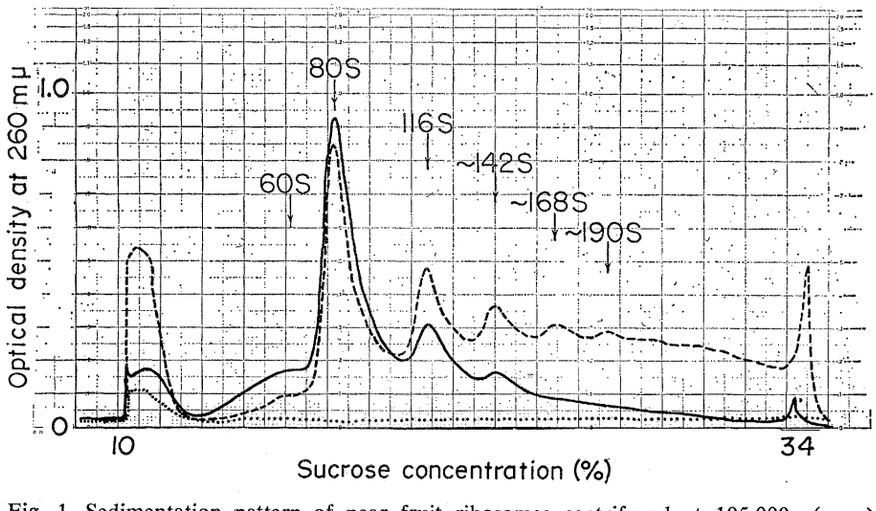

Fig. 1. Sedimentation pattern of pear fruit ribosomes centrifuged at 105,000*g* (——) in sucrose gradients after clarification and removal of the fraction sedimenting at 78,000*g* (- - - -). Omission of polyvinylpyrrolidone (PVP) from the isolation medium precludes recovery of ribosomes (. . . . .). The sucrose gradient (10 to 34 percent weight per volume) was centrifuged for 4 hours at 24,500 rev/min.

to neutralize vacuolar acids as determined by titration of a representative sample. Cysteine and PVP have been used in the isolation of active mitochondria from fruit tissues (*6*); they prevent browning and mitigate the detrimental effects of phenolic substances. Maceration was continued for 30 to 40 minutes as the powdered frozen homogenate completely thawed. As portions of the homogenate melted, the *p*H was monitored and adjusted with 1*M* KOH. Ribosomes were obtained with standard differential centrifugation techniques (*7*) and examined by centrifugation in sucrose density gradients (SDG), electron microscopy, and ultracentrifugation (*8*).

As revealed by SDG centrifugation (Fig. 1), several ribosomal components, including 60*S* subunits, 80*S* monomers, and two or more polymeric forms, were obtained from pear fruit. In Fig. 1, the solid line represents a standard ribosomal preparation sedimenting at 105,000*g*. A proportionately larger number of polysomes sediment during the intermediate centrifugation (78,000*g*) or during subsequent clarification at the same speed. When pellets obtained from intermediate centrifugations were thoroughly resuspended and clarified at 30,000*g*, the supernatant fraction contained ribosomes predominantly in monomer and polymeric forms (Fig. 1). Ribosomes could not be isolated from a pear preparation that was similar in all respects except for the omission of PVP. Omission of cysteine did not affect the physical properties of the ribosomes. Its use as a precautionary measure may be more pertinent when ribosomal activity is examined.

The structure of pear ribosomes depends upon Mg^{++} (Fig. 2). Breakdown of the monomer into 42 and 60*S* subunits clearly increases as the concentration of Mg^{++} is lowered from 6 to 0.5 m*M*. A qualitatively similar but less extensive breakdown of the polysomes occurs when the *p*H is less rigorously controlled during maceration.

Corroborative sedimentation values for several polysomal fractions were determined with the schlieren optics of a Spinco Ultracentrifuge. Evidence that polymers were attached to messenger RNA and not to mere random aggregates was obtained by treatment of a ribosomal suspension with 1 μg of ribonuclease per milliliter (Worthington Biochemical Corp., Freehold, N.J.) for 10 minutes at 25°C prior to centrifugation in density gradients. Such treatment results in an almost total loss of polymeric forms with a commensurate increase in monomers. At higher concentrations of ribonuclease (10 μg/ml) the monosomes are also broken into lighter fractions.

We prepared ribosomes to be examined by electron microscopy by adding a 10 percent formalin solution to fractions from an SDG zone and dialyzing the preparation for 4 to 8 hours at 0° to 2°C against a solution of 0.01*M* potassium phosphate buffer (*p*H 6.5) and 6 m*M* $MgCl_2$. The ribosomes were placed on a formvar-coated grid and observed with an RCA EMU-3 electron microscope; uranium was used for shadowcasting. Figure 3 shows pear ribosomes from the SDG zones containing monomers (A) and trimers (B); monomers predominate in the 80*S* fraction (Fig. 3A), while several polymeric forms are seen in the 142*S* fraction (Fig. 3B).

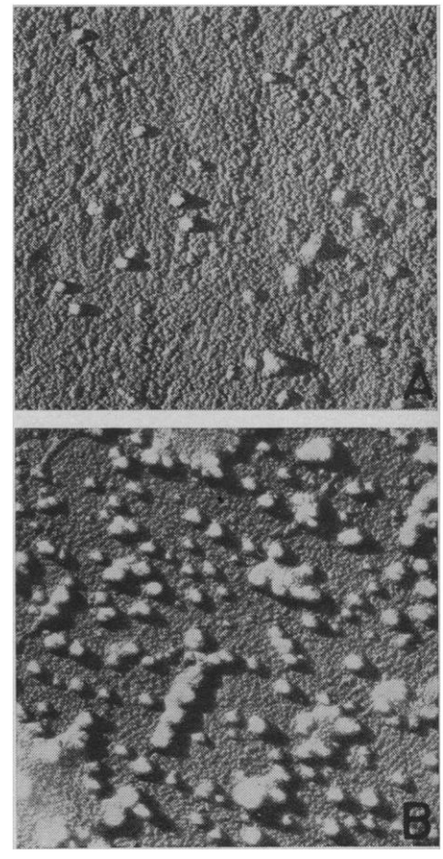

Fig. 3. Electron micrographs of pear fruit ribosomes from the 80*S* (A) and 142*S* (B) regions of the sucrose gradient (× 44,000).

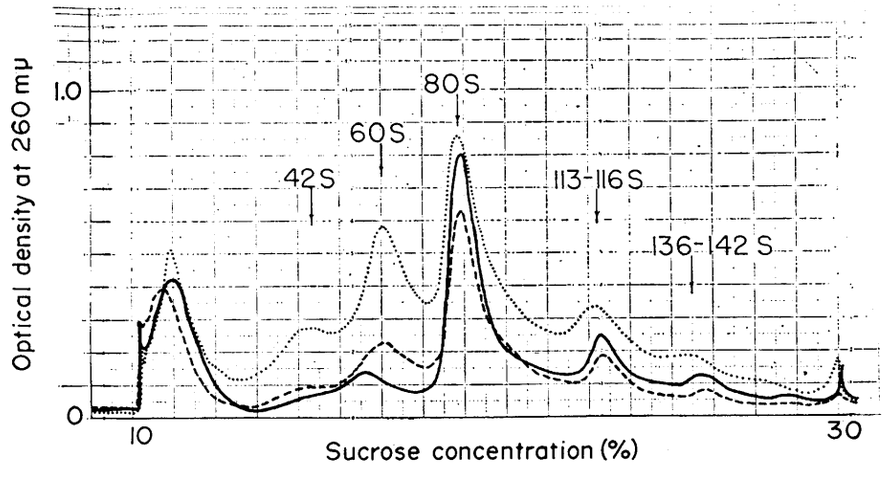

Fig. 2. Sedimentation patterns in sucrose gradients illustrating the dependency of pear ribosomes on Mg^{++} concentration. A proportionate increase in 42*S* and 60*S* subunits occurs as the concentration of Mg^{++} in the isolation medium and sucrose gradient is lowered from 6 m*M* (———) to 2 m*M* (- - - - -) and 0.5*M* (. . . . .). The sucrose gradient (10 to 30 percent weight per volume) was centrifuged for 6 hours at 24,500 rev/min.

Ribosomes isolated from pears and apples (not shown), having in common the physical properties described for similar preparations from other tissues, should prove functional in protein synthesis and useful in the study of aging and responses to massive irradiation at the molecular level.

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7. Ribosomes were obtained with the following

sequence of centrifugations: the homogenate was centrifuged at 30,000*g* for 15 minutes; supernatant fraction at 78,000*g* (Spinco Rotor 30) for 30 minutes; and the supernatant fraction at 105,000*g* (Spinco Rotor 40) for 2 hours. The pellet was resuspended in 1 to 2 ml of media containing sucrose and $MgCl_2$ in phosphate buffer with a motor driven pestle for 10 minutes; it was then clarified at 78,000*g* for 15 minutes. All isolation steps were carried out at 0°C. The resulting ribosomal suspension (0.5 ml) was placed on linear sucrose gradients prepared in Spinco SW 25.1 rotor tubes by the method of Britten and Roberts [*Science* **131**, 32 (1960)], and the tubes were centrifuged at 24,500 rev/min for 3 to 6 hours. All gradients contained 0.01 *M* potassium phosphate buffer (*p*H 6.4) and 6 m*M* $MgCl_2$ unless otherwise indicated. Optical density at 260 mμ was recorded continuously as the gradient solution was pushed out of the centrifuge tube with a sucrose solution of higher density and through a micro flowcell in a Beckman DU spectrophotometer.

8. We thank Dr. R. Doi and Dr. T. Hsiao for valuable suggestions, Dr. R. Criddle for the ultracentrifugations, J. Pangborn and K. Miyano for electron microscopy, and K. Fisher for technical assistance. This study was supported in part by AEC contract AT(11-1)-34, P.A. 112, Report No. UCD-34P112-27. L(L)K receives a graduate assistantship under the same contract.

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Navigation of Single Homing Pigeons: Airplane Observations by Radio Tracking

Abstract. *Navigation of homing pigeons was investigated by tracking their homeward flights from a light airplane. Released on successive days from a single training point 35 miles (56 kilometers) from home, individual pigeons, each carrying a transmitter, were repeatedly tracked back to their loft. No two tracks covered the same ground for even short distances, yet all tracks were within 10 miles of a straight line. Results from further releases north and south of the training point suggest that pigeons often use three methods in sequence to find home: compass orientation, bi-coordinate navigation, and orientation by familiar landmarks.*

How homing pigeons find their lofts is still obscure despite a long history of investigations (*1*). Since the work of Kramer and Matthews (*2*), it has been widely held that pigeons have the ability to navigate from any release point back to their home loft. Until now, however, there has been little direct information on the behavior of individual pigeons on their homeward flights. The visual following of flocks of pigeons from light aircraft by Griffin, Hitchcock, and Yeagley (*3*) yielded some data, but flock behavior is always some unknown consensus of the reactions of individual birds and is thus difficult to analyze meaningfully. Visual tracking of single pigeons was so difficult that they were not tracked far enough to provide much information. With accumulation of new data and new hypotheses (*4*), it has become increasingly important to know what paths single pigeons follow on their homeward trips. Radio tracking from light aircraft offered a way to obtain this information.

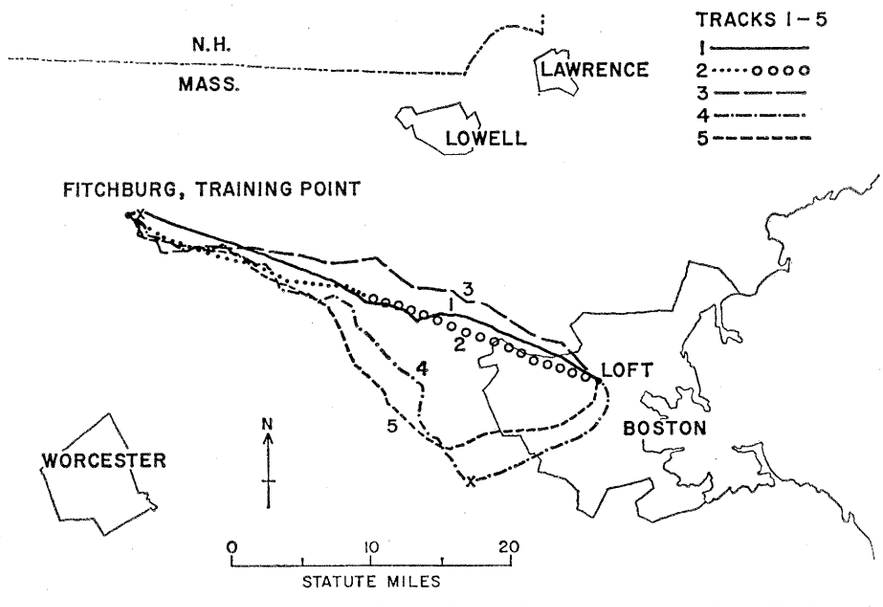

Fig. 1. The five consecutive tracks of Blue from Fitchburg made on the 16th through the 20th releases from the same place. Track No. 6, the 21st release from Fitchburg, was incomplete and is therefore omitted. Releases from Fitchburg in 1964: Track No. 1, 14 June, the 16th release; track No. 2, 18 June, the 17th; track No. 3, 19 June, the 18th; track No. 4, 23 June, the 19th; and track No. 5, 25 June, the 20th release. Sitting places are indicated by ×; open circles indicate area where bird was not followed.

During the summers of 1964 and 1965 we tracked ten individual homing pigeons on 131 flights from various release points to our Cambridge, Mass., loft. Each pigeon carried a 30-g, 52-Mcy transmitter, whose signal was followed with receivers in the airplane (*5*). We initially trained the pigeons by releasing them at increasing distances WNW of the loft until they had been released repeatedly from Fitchburg, Mass., 35 miles (56 km) WNW of the loft; and we compared the returning times of birds in these three categories: (i) birds with no load, (ii) birds with 2-g harness, and (iii) birds with harness plus a 28-g transmitter. On the average, birds wearing harness or harness plus transmitter homed more slowly than those with no load; yet the fastest homing times (when the birds flew home directly) in all three categories were comparable (30 to 40 mi/hr or 48 to 64 km/hr, average airspeed). Birds wearing harnesses were more likely to sit at the release point, but when they flew, their airspeed was the same as that of unharnessed pigeons. Loads up to 40 g seemed to have no effect on the pigeons' behavior aside from that caused by the harness alone.

Figure 1 shows five tracks made by Blue (color of the pigeon) on the 16th through the 20th releases from the Fitchburg training point. These were the only training releases of this pigeon that were followed. Four other birds were tracked on 31 of their releases, and Blue's five tracks are typical examples. The greatest deviation of any of the 36 tracks was 10 miles from a straight line connecting Fitchburg and Cambridge over the 35-mile course. The tracks in Fig. 1 show abrupt turns and the places where Blue sat. In all Fitchburg tracks, each bird seemed to start home most frequently when the airplane was farthest from the sitting place, which suggests that the tracking aircraft was disturbing the pigeon's flight. During these trackings, as with other Fitchburg flights, the airplane frequently came within 1/2 mile, or passed in front, of the pigeons, and, as seen on 20 occasions, the birds tried to avoid the plane by making