

region of rapidly sedimenting material. There was approximately 3 percent of such material in the treated sample. The shape of the curve from a graph of \bar{S} plotted against total protein concentration depends on the distribution of particles according to size. If the distribution depends on concentration, there is an increasingly larger fraction of the lower molecular weight species at low concentrations. A linear relationship, a result incompatible with rapidly reversible interactions, existed. It should be emphasized that aggregates removed from the boundary region and which are not optically recorded are not included in the calculation of the weight-average sedimentation coefficient. Convincing qualitative evidence for highly aggregated material present in the treated bovine serum albumin but absent in the untreated was obtained by sedimentation in the ultracentrifuge with the "field relaxation method" (14).

Solutions of treated and untreated bovine serum albumin were dialyzed separately against equal volumes of 0.1N KCl in an attempt to demonstrate low molecular weight fragments resulting from treatment with high-frequency sound. The absorbency of the dialysates from the two solutions measured in the region of 250 to 280 m μ was identical. Peptides were not detectable in the dialysates by the Lowry procedure (10). The identical conductivity observed in solutions of treated and untreated bovine serum albumin provides further evidence for the absence of low molecular weight fragments. When samples of treated and untreated albumin were centrifuged for 60 minutes at 105,000g, within the limits of sampling errors no difference in concentration distribution was observed, nor was there a detectable amount of sediment at the bottom of either tube.

Native bovine serum albumin is a compact relatively symmetrical molecule (15) containing either 17 or 18 disulfide groups (16). If no change in molecular weight is assumed, it is difficult to imagine any change in configuration which would substantially increase the capacity of the bovine serum albumin molecule to scatter light. The most plausible explanation for the effects of high-frequency sound appears to be aggregation. The protein forms aggregate in acid solution (17), on denaturation with urea (18), and on heating (19) owing to polymerization—one interpretation—by means

of sulfhydryl-disulfide interchange. As ordinarily prepared, 68 percent of the bovine serum albumin molecules contain a free SH group (16). A second possibility, discussed by Waugh (20), is the reversible formation of fibrous aggregates. Since oxygen was not excluded, aggregation resulting from oxidation by free radicals formed during the sound treatment (8) provides a third plausible mechanism.

In a mixture of dimers and monomers, 62 percent of dimers would be required for a weight-average molecular weight of 110,000, a value inconsistent with the results from ultracentrifuge measurements. With a sample containing 59 percent dimers, Bro, Singer, and Sturtevant (17) observed a distinctly bimodal boundary. A polymer having an average weight of 1×10^6 , comprising 2 to 4 percent of the treated sample, is consistent with our observations. A portion of the aggregated material was removed from the boundary region and was not optically recorded in sedimentation-velocity studies. Other procedures employed in studying the treated samples did not remove these larger aggregates from consideration, and the results reflect the presence of aggregates. The small shoulder, amounting to less than 5 percent of the total area, seen in the electrophoretic patterns would appear to represent some polymer formed during the exposure to high-frequency sound. Extensive fragmentation, even where reaggregation of fragments occurred, would be expected to result in distinct and more striking qualitative differences in ultracentrifugal and moving-boundary electrophoretic properties.

The changes in the optical rotatory properties of the treated albumin were slight enough to be considered within experimental error. The data shown in Table 1, however, are the average of a number of experiments. Because the specific rotation in all cases decreased and the dispersion constant (λ_0) was greater after treatment, we are led to conclude that the small changes observed were indeed real. Steinrauf and Dandliker (19) observed similar small changes in optical rotation, $[\alpha]_D$, during the polymerization of bovine serum albumin with heat.

EUGENE L. HESS
PAUL W. L. CHUN
R. L. CROWLEY

Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts

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Separation of Microsomal RNA into Five Bands during Agar Electrophoresis

Abstract. *Microsomal RNA from rabbit livers and lymph nodes separate into five major bands during agar-gel electrophoresis. The electrophoretic method may be used either as an analytical or preparative tool. The 33S and 19S peaks of microsomal RNA from sucrose-gradient zone centrifugation divide into two bands each during simple agar electrophoresis.*

The simple technique of electrophoresis in agar devised by Gordon (1) has served to identify, separate, and prepare many substances from complex mixtures. Although Uriel *et al.* (2) showed RNA electrophoresis in agar and that RNA could be identified by pyronine staining, Bachvaroff, Yomtov, and Nikolov (3) first applied the method to the separation of RNA into

fractions. They found that RNA extracted from the whole rabbit spleen could be resolved into five bands in simple agar electrophoresis. This study compares electrophoretic fractionation of microsomal RNA with that obtained by zone centrifugation in a sucrose gradient.

Microsomal and cell-sap RNA (soluble RNA) were prepared from livers and lymph nodes of adult rabbits as described by Sporn and Dingman for rat liver (4), except for two alterations. The tissue was homogenized with a Potter Elvehjem teflon grinder, and the cell-sap fraction was centrifuged for 11 hours at 105,000*g* to sediment the remaining microsomal RNA. The optical absorption of these RNA preparations in the native state was measured at 230, 260, and 280 $m\mu$ in 0.136*M* potassium phosphate buffer, pH 7.0. The absorption ratios, 260:230 and 260:280, respectively, were 2.15 and 2.10 for

microsomal RNA and 1.86 and 1.99 for cell-sap RNA. The protein contamination of microsomal RNA, as measured by the method of Lowry *et al.* (5), was 1.6 percent.

Zone centrifugation was performed by the method of Martin and Ames (6) and the results were analyzed as described by Sporn and Dingman (4). For this purpose 0.5 ml of microsomal RNA in 0.1*M* potassium acetate buffer, pH 6.0, was layered on a 30-ml linear (4 to 20 percent) sucrose density gradient containing the same buffer. After centrifugation in the swinging-bucket rotor (SW-25.1) for 16 to 18 hours at 25,000 rev/min at 4°C, the tubes were punctured, and 1-ml fractions were collected. A typical sedimentation analysis of microsomal and cell-sap RNA from rabbit livers is shown in Fig. 1 (upper), along with the sedimentation coefficients of the material at the peaks. The results, 33*S* (± 1), 19*S* (± 1), and 6*S* (± 1.5), agree well with those of Sporn and Dingman for RNA from rat livers.

For electrophoresis, 0.2 to 0.4 ml of RNA (2 to 10 mg/ml) was precipitated with three volumes of alcohol. The precipitate was dissolved in 0.2 ml of veronal buffer, pH 8.2, 0.05*M*, and the solution was mixed with an equal volume of 2 percent melted agar. The mixture was placed in a hole or trough cut out of the agar gel of the electrophoretic plaque which was 12.5 cm long. The agar gel of the plaque was composed of 1 percent agar (Difco) in 0.05*M* veronal buffer, pH 8.2. Electrophoresis was conducted for 2 hours at 4.5 v/cm. After the plaques were fixed, dried under filter paper, and stained with pyronine according to the procedure of Uriel *et al.* (2), the bands became visible.

Groups of appropriate successive tubes from the density gradient were mixed and an aliquot of each pool was subjected to electrophoresis in a gel plate together with a sample of whole microsomal RNA, cell-sap RNA, and RNA digested with ribonuclease for comparison.

A drawing of the stained electrophoretic plate (Fig. 1, bottom) indicates which tubes were included in each fraction. In all cases the electrophoretic mobility of the RNA from the density gradient increased in inverse proportion to the sedimentation rate. Both the 33*S* and 19*S* peaks appeared to become resolved into two components each. However, the 6*S* peak remained intact and migrated with a velocity very nearly as great as cell-sap RNA.

During electrophoresis whole microsomal RNA was resolved into five major bands, identified in Fig. 2 (top) as A to E. To verify that the bands A and B, C and D, and E properly represented materials with sedimentation velocities of 33*S*, 19*S*, and 6*S*, respectively, the experiment was reversed. A fresh sample of microsomal RNA was mixed with agar and placed in a trough in an electrophoretic plate. After 2 hours of electrophoresis, strips of gel 0.5 cm wide, parallel to the starting trough, from that locus to the anode were cut out of three-fourths of the plate. In order to determine which strip contained which of the bands (Fig. 2, A-E), the uncut portion of the plate was stained. The RNA was then eluted from the appropriate gel strips by freezing and thawing. After the RNA was precipitated from the eluate with three volumes of alcohol and dissolved in 0.1*M* acetate buffer, pH 6, each fraction was individually analyzed by zone centrifugation. The sedimentation velocities of RNA from bands A and B were 33*S* whereas those from bands C and D were 19*S* and that of E was 6*S* (Fig. 2, bottom).

The electrophoretic pattern of microsomal RNA from liver was not unique;

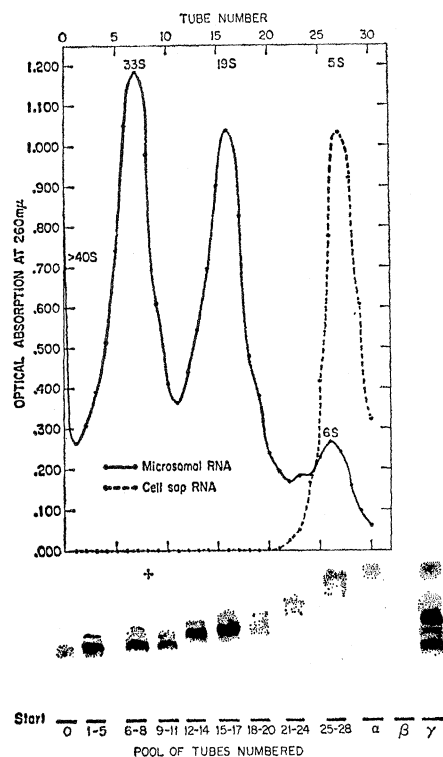


Fig. 1. (Top) Sedimentation analysis of rabbit liver microsomal and cell-sap RNA. The tubes contain successive 1-ml fractions collected from the density gradient. The sedimentation coefficients are given above each peak. (Bottom) Electrophoresis of RNA (stained) showing the (upward) migration toward the positive pole from the start. Each dash represents one starting position. The numbers under each dash show which tubes from the density gradient were pooled prior to electrophoresis. The letters α , β , and γ show the position of cell-sap RNA, microsomal RNA digested with ribonuclease, and microsomal RNA, respectively.

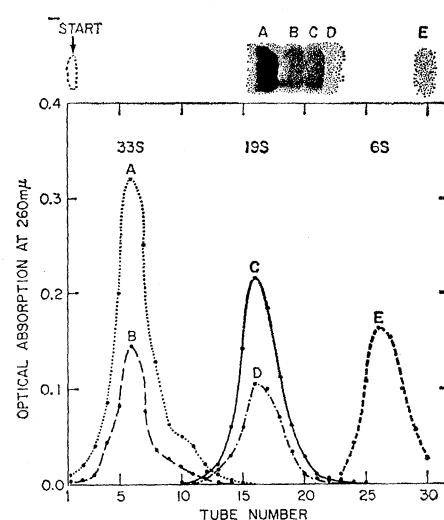


Fig. 2. (Top) Stained portion of electrophoretic plaque of electrophoresis of microsomal RNA; migration horizontally to the right toward the positive pole. The five major bands of the microsomal RNA are labeled by the superscripts A-E. (Bottom) Zone centrifugation analysis of each band of RNA eluted from the gel after electrophoresis. The tubes represent successive 1-ml fractions from the density gradient. The letters A-E over their respective lines show which curve resulted from the sedimentation analysis of the corresponding band. The calculated sedimentation coefficients are given above the peaks of the curves.

microsomal RNA from rabbit lymph nodes yielded a similar, if not identical, pattern.

Whether the division of microsomal RNA into five electrophoretic bands represents a separation of intrinsically different kinds of RNA, or an artifact of the method, remains to be determined. Analyses of the RNA in each band to ascertain the base composition, secondary structure, and biological activity should resolve this issue.

RADOSLAV BACHVAROFF*

PHILIP R. B. MCMASTER†

Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

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7. We are indebted to Dr. M. Landy for his helpful suggestions.
- * Guest Scientist at the National Institutes of Health from the Research Institute for Epidemiology and Microbiology, Boul VI, Zaimov 26, Sofia, Bulgaria.
- † Present address: Laboratory of Germfree Animal Research, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland.

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First Linkage of a Species Antigen in the Genus *Streptopelia*

Abstract. Twenty-two progeny in a single backcross family of a species hybrid between the dwarf turtle dove and the ring-neck dove exhibited strong association in a repulsion phase of linkage between the silky feather trait and the species-specific hu-8 blood character. This family constituted a test cross for six characters: sex, peanut reactivity, silky feathering, and the three independent species-specific blood characters, hu-1, hu-4, and hu-8.

Linkage of genes for individual blood group traits with similar genes or those for other genetic traits has been reported in the rabbit (1), in man (2), and in chickens (3). Such studies in other species are rare (4). An especially productive species hybrid in doves has permitted a new test of linkage of blood groups.

A dark, silky female hybrid was produced in a cross of a male dwarf turtle dove, *Streptopelia tranquebarica*, sometimes called *humilis*, with a female, silky blond ring-neck dove, *Streptopelia risoria*. This hybrid produced 22 progeny in a mating with a male blond ring-neck dove. The family constituted a test cross in which the female was segregating for six genetically determined character pairs: that is, for the sex chromosome, Z or no Z; for feather condition, silky or normal; for peanut reactivity, positive or negative; and for hu-1, hu-4, and hu-8 species blood types, presence or absence.

The three sex-linked color alleles, dark, blond, and white, in order of decreasing dominance, have been described by Cole (5). Therefore, the backcross of this dark hybrid female to a blond male constituted a sex-linked mating in which the sex of the progeny was accurately inferred by their color (males had dark plumage, females had blond or white plumage) and later veri-

fied in the mature progeny by actual matings or by the method of Miller and Wagner (6). The silky trait of the plumage is a "partial dominant" (7). The reactivity of the red cells with peanut extract is a recessive trait in ring-neck doves (8). The red cells of all five dwarf turtle doves tested, two males and three females, were nonreactive with peanut extract.

The so-called "species-specific" antigens of the erythrocytes detected by agglutination tests and analyzed from several crosses have been made by Irwin and co-workers. The details of the immunological procedures have been described many times (9). The dwarf turtle dove has three such species-specific antigens in contrast to ring-neck doves, and the antigens were designated hu-1, hu-4, and hu-8 by Stimpfling and Irwin (10). Reagents detecting these three types of cells may be prepared in several ways; but originally, such reagents were made by producing antisera in rabbits against the red cells of one parental species (that is, dwarf turtle dove). After complete absorption of the antisera with the red cells of ring-neck doves plus those of one or more backcross hybrids possessing all but one of the antigens, various reagents are obtained which are specific for each of the cell types, hu-1, hu-4, or hu-8. Such reagents were made for the present

tests, and, they were tested in parallel with reagents provided by M. R. Irwin so that continuity of nomenclature would prevail.

The two species, *S. risoria* and *S. tranquebarica*, definitely are considered to be different species (11). Indeed, *S. tranquebarica* is the only member of the genus that shows distinct sexual dichromatism (12). Nevertheless, reproductive success in this backcross family was excellent.

In Table 1, the phenotypes of the backcross progeny directly indicate the genetic contribution of the F₁ hybrid. For example, the first offspring received from her a sex chromosome, a gene for lack of peanut reactivity, a gene for hu-1, genes for the ring-neck alternative to hu-4 and to hu-8, and a gene for silky feather condition. While, in contrast, the third offspring received no Z chromosome from the hybrid, but did receive a gene permitting peanut reactivity, the ring-neck dove's alternatives for hu-1 and for hu-4, the dwarf turtle dove gene for hu-8, and a gene for normal feathers.

It is evident in Table 1 that, in this mating, hu-8 and the silky trait have not yet occurred together in an offspring, and with one exception, all progeny without hu-8 were silky. Of course, the chi-square value is very highly significant. The exceptional offspring with normal feathers and lacking hu-8 presumably represents a crossover. The linkage value, therefore, likely would approximate 5 percent.

Table 1. Recombination of characters in sequential progeny from a test cross of a silky hybrid female (*S. tranquebarica* × *S. risoria*).

Sex	Peanut extract	Blood type	Feather condition
♂	—	1	S*
♀	—	8	N†
♀	+	8	N
♂	+	4	S
♀	+	1 4	S
♀	—	4	S
♂	—	1 4 8	N
♀	+	8	N
♂	+	1 4 8	N
♀	+	1	4
♀	—	1	4
♂	—	8	N
♀	+	1	4
♀	—	1	4 8
♂	—	1 4	N
♂	—	4 8	N
♀	+	1 4	S
♂	+	1 4	S

*S = silky feathers. †N = normal feathers.