virus. The cultures were examined 3 days later. The appearance of a cytopathogenic effect due to Coxsackie A-9 virus was interpreted as indicating neutralization of the interfering effect of the rubella virus.

Only the packed-cell antigens had demonstrable antigenicity in the complement-fixation tests. The highest titers of antigen were prepared from cells harvested 2 days after inoculation of RK-13 cultures and 7 to 10 days after inoculation of primary cultures of kidney from African green monkeys (Table 1). The serum used for these tests was the first specimen with a high titer obtained from a 28-year-old patient from Maryland 1 month after the rash appeared (Table 2).

When tested against serum specimens from individuals with rubella, the titers of complement-fixing antibody paralleled, but were generally lower than, the neutralizing antibody titers (Table 2). The time of development of complement-fixing antibody was slightly later than that of neutralizing antibody since in none of four cases was there definite evidence of the development of complement-fixing antibody during the time of rash. Complement-fixing antibody persisted for at least 8 months, but was detectable in only four of 12 specimens obtained from normal individuals and pregnant women who had the disease 10 to 20 years previously; however, all 12 specimens contained neutralizing antibdy. There was no evidence of heterologous reaction with the seven other myxoviruses tested (Table 3). Paired serum specimens from monkeys which were experimentally infected with rubella in a previous study showed the development of CF antibody in the serum specimens taken one month after the intranasal inoculation of the virus (*2*).

Several facts concerning the packed-cell complement-fixing antigens emerged from these investigations. First, the titer of the virus inoculated in the cultures for antigen production should be no less than the titers we used; with smaller quantities of virus, only low titers of antigens could be produced. Second, the time of maximum production of complement-fixing antigen differed for the cell lines used. Similar cell-associated antigens were prepared with other tissues which support the growth of the virus, including $LLCMK_2$ cells and the chronically infected RCC-$LLCMK_2$ cells (*3*). In each case, the day on which the maximum amount of complement-fixing antigen was produced differed but was within the range of 2 to 10 days after inoculation. Third, complement-fixing antibody persisted for a least 8 months but diminished after 10 to 20 years. This differs from neutralizing antibody which persisted for many years (*10*). The presence of complement-fixing antibody in 4 of 12 patients who had neutralizing antibody but no known recent rubella is probably due to the continuation of complement-fixing antibody in these individuals for a long period of time; however, it may indicate relatively recent infections which were not recognized by the patients, or infection with an antigenically related virus.

The complement-fixation test has direct clinical application since it can provide rapid serological confirmation of the diagnosis of rubella. The specificity of the test should make it particularly useful for epidemiological investigations including large-scale studies of the efficacy of gamma globulin and new vaccines in the prevention of disease and the prevention of possible damage to the foetus. Antibody tests such as virus neutralization may still be necessary to identify patients who experienced rubella a number of years before being studied.

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8. For the RK-13 rabbit kidney cells, the growth medium consisted of Medium 199 and calf serum, 95 and 5 percent, respectively. For the maintenance medium, the calf serum was reduced to 2 percent. The cells were incubated at 34°C, and were inoculated with 4 to 6 ml containing 10^4 to 10^6 virus per 0.2 ml. The virus had been grown in RK-13 cells or in kidney tissue from the African green monkey. The growth medium for the kidney tissue from the African green monkey consisted of Eagle's medium, 89.8 percent; calf serum, 10 percent; SV-5 antiserum, 0.2 percent. The maintenance medium consisted of Eagle's basal medium, 99.8 percent; and SV-5 antiserum, 0.2 percent. The cells were incubated at 36°C, and were inoculated with 4 to 8 ml containing 10^3 to $10^{4.5}$ virus per 0.2 ml, the virus having been grown previously in kidney tissue from the same species of monkey.
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Density-Gradient Centrifugation: Non-Ideal Sedimentation and the Interaction of Major and Minor Components

Abstract. *A small amount of southern bean mosaic virus was contained in a narrow zone after density-gradient centrifugation, but in a much wider zone after centrifugation with a large amount of a second virus. Zone-spreading of a major component by non-ideal sedimentation in density-gradient centrifugation can cause zone spreading of a minor component that the major component overlaps.*

The width of a zone of virus or protein after sucrose density-gradient centrifugation increases as the amount in the zone increases (*1*). The amount of virus or protein in a zone of a given width is only a few percent of that which would be present if the negative density-gradient which is due to the virus or protein concentration gradient were equal to the positive density gradient due to the sucrose concentration gradient. Brakke (*1*) postulated that the increase in zone width as the amount of virus or protein in-

creased was due to non-ideal sedimentation, which became increasingly important as the concentration of virus or protein increased. He further postulated that the mechanism of non-ideal sedimentation was the sedimentation of volume elements containing such excess virus or protein particles as were expected from statistical or fluctuation theory. If this mechanism of non-ideal sedimentation is correct, then the spreading of a component present in high concentration should result in the spreading of a component present in low concentration if the two zones overlap. The present experiments were designed to test this hypothesis.

Radioactive southern bean mosaic virus (SBMV-C^{14}) (AC No. 17) (*2*) was prepared by growing infected Bountiful bean plants (*Phaseolus vulgaris* L.) in an atmosphere containing $C^{14}O_2$. The SBMV-C^{14} was purified by differential centrifugation after heating the extract from the infected plants for 15 minutes at 50°C to denature the normal plant proteins. Tobacco mosaic virus (TMV) (AC No. 2) was purified by the method of Boedtker and Simmons (*3*). Brome mosaic virus (BMV) (AC No. 66) was purified by differential centrifugation of a frozen extract of diseased barley, *Hordeum vulgare* L. Moore.

The zone width of SBMV-C^{14} was determined after density-gradient centrifugation of a small amount of SBMV-C^{14} by itself or in the presence of a large amount of either BMV or TMV. Gradient columns were prepared by layering 4,7,7, and 7 ml of 0.01*M* phosphate buffer, *p*H 6.8, containing 100, 200, 300, and 400 mg of sucrose per milliliter, respectively, in the centrifuge tube. The sucrose column was allowed to stand overnight, 2 ml of virus solution was then floated on it, and it was centrifuged for 3 hours at 23,000 rev/min at 5°C (SW25.1 rotor of the Spinco model L ultracentrifuge) (*4*).

The centrifuged gradient columns were examined to obtain a recording of absorbancy at 253.6 mμ at all depths (ISCO Density-Gradient Fractionator) and consecutive fractions were collected (*5*), 0.2 ml at depths where preliminary experiments had indicated that most of the radioactivity would be found and larger samples at other depths. These samples were diluted with four volumes of water and three 0.2-ml portions of each were transferred to glass planchets containing lens paper. The weights of material on each planchet were obtained in order to correct for self-absorption.

Because of only slight differences in total amounts of sucrose in the samples, no self-absorption correction was necessary in presenting data (see Fig. 1) for radioactive zones. In order to estimate recovery of viruses on the gradient columns, it was necessary to correct for differences in self-absorption between the original samples of radioactive virus and each radioactive fraction. High concentrations of sucrose in the samples resulted in erratic counts apparently caused by the geometric effects of sucrose crystallization. The above fourfold dilution eliminated this source of poor reproducibility.

Each gradient column of the experiment whose results are shown in Fig. 1 contained 0.3 mg (42,000 count/min) of SBMV-C^{14}. In addition, the second column contained 40 mg of TMV and the third, 20 mg of BMV. The BMV has $S_{20,w}$ of 86*S* (*6*); SBMV, of 115*S* (*7*); and TMV, of 187*S* (*8*). These three viruses are well separated by density-gradient centrifugation of small amounts. However, the zones of large amounts of BMV and TMV as used here are wide and overlap the SBMV zone.

Apparently the SBMV-C^{14} zone was much wider in the presence of large amounts of the other two viruses than

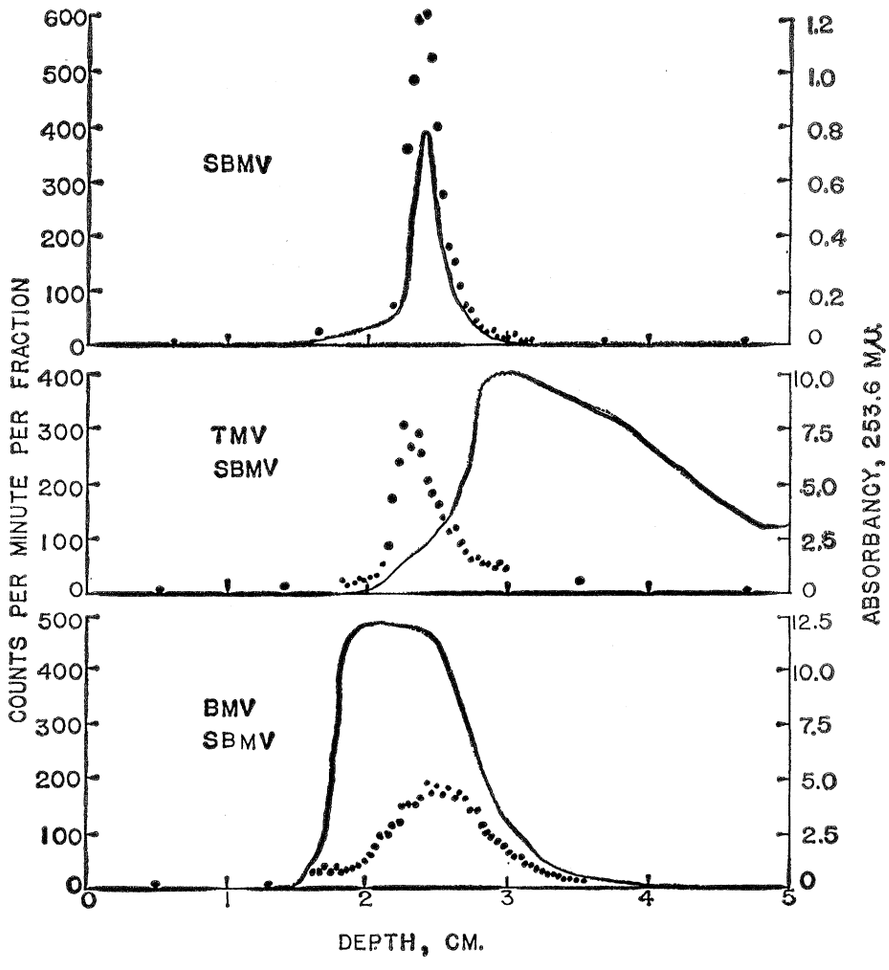

Fig. 1. Absorbancy and radioactivity at different depths after density-gradient centrifugation of 0.3 mg of SBMV-C^{14} by itself, or in the presence of 40 mg of TMV or 20 mg of BMV. A cell with a 2-mm light path was used and absorbancies were multiplied by 5 to give values for a 1-cm light path. The absorbancy in the scanning pattern of SBMV-C^{14} alone was expanded electronically to increase sensitivity. The recovery of radioactivity was 106 percent for the column with SBMV only, 92 percent for SBMV plus TMV, and 94 percent for SBMV plus BMV, after correction for differences in self-absorption between the original sample and samples obtained from the sucrose-gradient column.

in their absence. Furthermore, the peak concentration of the SMBV ($-C^{14}$) was always lower in the tube in the presence of BMV than when SBMV(C^{14}) was sedimented by itself or with TMV. These data show that non-ideal sedimentation of a major component during density-gradient centrifugation can result in widening of the zone of a minor component that it overlaps and in a different apparent sedimentation rate of the minor component.

Sucrose density-gradient centrifugation is often used to determine the size of particles carrying a certain biological or chemical activity. Results in such experiments are obtained by determining the activity, for example, virus infectivity, enzymatic activity, or a radioactive tracer, at various depths in the column after centrifugation. Viruses and proteins form wide zones at high concentrations (*1*). Apparently non-ideal sedimentation, which causes the zone spreading, is pronounced at concentrations higher than 2 to 3 mg/ml and the sedimenting material spreads until the maximum concentration does not exceed 2 to 3 mg/ml. Our studies show that it is not the concentration of an individual component that determines the amount of non-ideal sedimentation and zone spreading, but rather the total concentration of materials sedimenting together. If this concentration is high, and followed by the determination of enzymatic or other activity, a particle present in low concentration will give neither a true indication of its homogeneity nor an accurate value of its sedimentation rate. For good resolution (*1*), the amount of sedimenting material should be less than 1 mg for the 2.5- by 7.6-cm tube of the SW 25 rotor of the Spinco model L.

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Countercurrent Multipliers in Avian Kidneys

Abstract. *The capacity to conserve urinary water by producing a concentrated urine is directly related to the number of Henle's loops in the kidneys of three terrestrial birds. This suggests that a Henle's loop countercurrent multiplier is responsible for urine concentration in these birds. Several features of the organization of the kidneys of these birds may account for the importance of multiplier number, as contrasted to multiplier length in mammals, in determining maximum urine concentration.*

Studies of salt and water balance in several races of Savannah sparrows and in the house finch showed that each of these taxa differs conspicuously in capacity to produce a concentrated urine (*1*). In addition, none of these taxa has functional salt glands (*2*). Therefore, a study of the renal and cloacal histology in representatives of these taxa was undertaken.

Two *Carpodacus mexicanus* (house finch), two *Passerculus sandwichensis brooksi*, and two *P. s. beldingi* (Savannah sparrows) were used. The drinking responses and urine-concentrating abilities of these individuals were typical for their taxa (*1*). The study showed that there are no gross differences in cloacal structure between taxa, but that there are differences in renal medullary development which are directly related to urine-concentrating ability. These differences involve the number and length of Henle's loops and therefore suggest that urine concentration is accomplished by a countercurrent multiplier system in the loop of Henle.

In birds, the medullary nephrons, characterized by the presence of Henle's loops, are scattered throughout the kidney lobes in groups called medullary lobules—that is, medullary cones (Fig. 1*A*) (*3*). There are several cortical lobules for each medullary lobule. These cortical portions, which consist of collecting tubules, and all parts of the nephron except the Henle's loop are analogous to terminal twigs in the dendritic organization within each kidney lobe. The medullary lobules are analogous to branches. Each medullary lobule unit is surrounded by a connective tissue sheath and can be recognized by a ring of collecting tubules which surrounds capillaries and thin segments of Henle's loops, and by concentric "layers" of thick segments of Henle's loops around the ring of collecting tubules (Fig. 1, *B* and *C*). The medullary lobules connect with ureteral branches which are analogous to limbs in the dendritic system. As a medullary lobule courses toward a ureteral branch, it often becomes contiguous with other lobules, forming a group of lobule units which is then enclosed in a common sheath of connective tissue (Fig. 1, *A* and *C*). Each lobule unit in the group becomes smaller as it approaches a ureteral branch because of a decrease in the number of Henle's loops (Fig. 1, *C* and D_{1-3}). Near the junction with a ureteral branch (Fig. $1D_3$) most collecting tubules have fused so that they are larger but fewer in number and only the longest Henle's loops are present. When all the collecting tubules from a lobule unit or group of lobules have fused, the resulting tubule is called a ureteral branch. These successively fuse until the trunk of the dendritic system, the ureter, is formed (Fig. 1*A*).

Because of these features of avian kidney organization, the average length of the Henle's loops in the kidney can be determined by averaging the number of concentric "layers" of thick segments of Henle's loops surrounding the ring of collecting tubules in each medullary lobule unit. An index of the number of Henle's loops in the kidney can be determined by averaging the number of medullary lobules in each kidney lobe since in birds only a few nephrons have Henle's loops (*4*). To obtain the average number of looped nephrons and the average length of the loops the relevant measurements were made in every fifth, transverse 8-μ section of the right and left pos-