The study does not define the genetic status of M.A.G. The fact that her enzyme activity falls between those of C.T. and C.T.'s parents could be due to various causes. Although nongenetic factors other than those already mentioned may play a role, the possibilities that multiple alleles or several genetic loci are involved should be considered. Regardless of the mode of inheritance, it is apparent that M.A.G. does not have sufficient cystathionine synthetase activity to prevent her from excreting homocystine in the urine.

The clinically more important question of why M.A.G. is not mentally retarded cannot be answered. Possibly her enzyme level, although low, was sufficient to permit normal mental development; perhaps environmental factors prevented the biochemical defect from causing mental retardation. It has been suggested that an adequate cyst(e)ine content of the diet during the neonatal period may alleviate clinical manifestations of the disease (*2*, *5*). During infancy, C.T. received only cow's milk, a relatively poor source of cystine. For the first 6 weeks of life M.A.G. received human milk, a richer source of cystine.

"Homocystinuria" has been proposed as the name of a disease (*2*). There is, however, a theoretical possibility that excretion of homocystine in the urine may have multiple etiologies; this possibility has already been considered in relation to patients described by Gerritsen and Waisman (*3*). Therefore, we suggest that the term homocystinuria be used to denote excretion of homocystine in the urine, without etiologic connotation. One of the diseases causing this manifestation is the inborn error of metabolism, cystathionine synthetase deficiency.

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Physical Interaction of a Murine Leukemia Virus with Influenza Virus in vitro

Abstract. *Incubated mixtures of PR8 influenza virus and Rauscher leukemia virus retained the egg infectivity and hemagglutinin of the influenza virus and the ability of the Rauscher virus to induce splenomegaly in mice. Density-gradient centrifugation on potassium citrate gradients revealed a new interviral product with an intermediate density as the principal constituent of such mixtures. Chicken erythrocytes adsorbed the Rauscher virus components as well as the influenza virus components of the interviral product from such mixtures at 4°C, whereas the Rauscher virus alone was little adsorbed. The adsorbed interviral product was eluted from the erythrocyte complex after incubation at 37°C.*

Infection of the same susceptible cell by different viruses can result in pronounced viral interference or synergism. Studies of these effects, however, often involve implicit assumptions that the viruses in the mixture necessarily retain their physical individuality, and that the biological consequences are initiated only on interaction with the cell. In any study of multivirus phenomena it is necessary to check the possibility that the different viruses may have mutually interacted before infecting the cell, and thus provided an effective new set of physical particles having altered biological behavior. This report presents evidence that the PR8 strain of influenza A virus and Rauscher murine leukemia virus readily interact physically in vitro to provide an interviral product having altered density and hemadsorption characteristics.

The Rauscher murine leukemia virus was prepared from the plasma of viremic Balb/c mice that had been previously inoculated with the virus; it was purified and concentrated by a series of differential centrifugations (*1*) so that 1 ml of the final viral concentrate in 0.05*M* sodium citrate solution (*p*H 6.75) was derived from 10 ml of the initial viremic plasma. Influenza virus was similarly isolated and concentrated from the allantoic fluid of embryonated hen eggs that had been inoculated with limiting dilutions of the virus and then incubated for 48 hours. Stocks of the viruses were stored in sealed ampules under nitrogen at −70°C. Before use, the thawed stocks were centrifuged for 2 minutes at 10,000*g* to remove any sediment.

Mixed at approximately 4°C, the viruses were incubated for 30 minutes at room temperature. Assays of the mixture revealed no significant change in the biological titers (egg infectivity and hemagglutinin of the input influenza virus, or the ability of Rauscher virus to cause spleen enlargement in Balb/c mice) compared with those of equivalent dilutions of the separate viruses (Table 1); at first glance no significant interaction of the viral components of the mixture was apparent.

Density-gradient centrifugation of the mixture revealed a somewhat different situation. The Rauscher virus alone, on density-gradient centrifugation for short periods in potassium citrate gradients, formed a single opalescent band (equilibrium density, 1.16 g/cm^3) containing the infectious virus (*2*). Preliminary experiments showed that on similar centrifugation the influenza virus stocks also formed a single opalescent band which coincided with the peaks of the influenza hemagglutinin and infectivity, but which had a distinctly different equilibrium density (1.20 g/cm^3). When, however, an incubated mixture of equal volumes of each virus was centrifuged on a shallow potassium citrate gradient, a new opalescent band appeared which had a density intermediate between those of the separate viruses (Fig. 1). This new intense band was considerably narrower (at 1 mm) than either of the opalescent bands (6 mm wide) formed on separate centrifugation of each virus singly in such identical gradients. Furthermore, the centrifuged mixture showed no significant opalescence in the region normally occupied by the influenza virus

alone. Some opalescence present in the density region corresponding to that normally occupied by the Rauscher virus was significantly reduced in intensity.

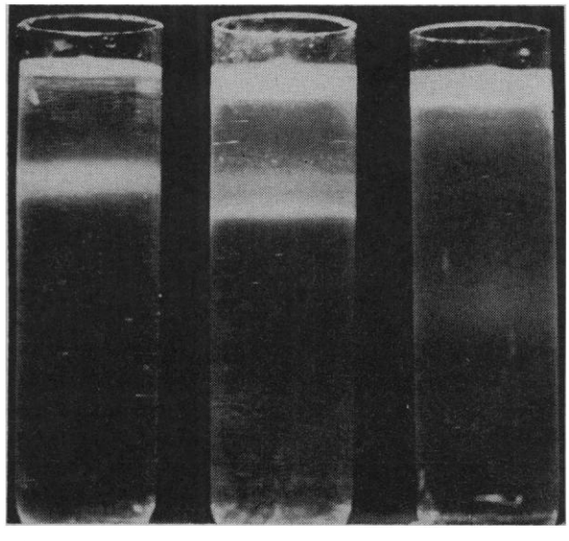
These findings indicated that simple mixture of the two viruses formed a new interviral product having an average density intermediate between those of the two viruses. The changes in opalescence (Fig. 1) suggested that the influenza virus of normal density was almost completely removed to form the new product, and that most of the leukemia virus was similarly removed, but that there was also an excess of normal Rauscher virus present.

Further experiments showed that the density of the band corresponding to an interviral product varied, within limits, with the relative concentrations of the two viruses in the mixture. Mixing the viruses in concentrations outside these limits, however, gave an excess of one of them, with only slight, if any, further change in the density of the new band.

The hemagglutinin content of fractions isolated from density-gradient centrifugations of the influenza virus alone, or of incubated mixtures of influenza and Rauscher viruses, was assayed. The density gradient of incubated mixtures showed a single sharp peak of hemagglutinin coincident with the new opalescent band, and only a low residual hemagglutinin at the position normally occupied by the influenza virus (Fig. 2). In three separate experiments quantitative recovery of the input influenza hemagglutinin was obtained at the new density position. This shift in hemagglutinin density in the viral mixture was accompanied by a corresponding density shift in influenza infectivity: a sharp peak of infectivity (infectious dose, ID_{50}, 8.6) coincided with the new opalescent band and only a low infectivity (ID_{50}, 4.3) remained in the region normally occupied by the free influenza virus. Assays of the density-gradient fractions for Rauscher virus infectivity showed that the density gradient of the incubated viral mixture contained a new sharp peak of infectivity which also coincided with the new opalescent band, and vastly reduced infectivity at the position normally occupied by the Rauscher virus alone.

The interviral product also formed when the viruses were incubated in phosphate-buffered (*p*H 7.0) saline (PBS), although the reaction did not quantitatively proceed quite so far; opalescence corresponding to some unreacted influenza virus was detected. The viruses were centrifuged (50,000*g* for 1 hour) from the citrate medium, and the pellet was suspended in the PBS prior to this interaction. Sucrose gradients were also used to demonstrate formation of the interviral product, but more prolonged centrifuging was required to attain equilibrium, and the opalescent bands were generally more diffuse on these gradients.

Interaction of each virus and of the incubated viral mixture with chicken erythrocytes was examined by direct observation and by density-gradient centrifugation of interaction samples on potassium citrate gradients after removal of the erythrocytes by low-speed centrifugation. As expected, the influenza virus alone readily agglutinated the erythrocytes at 4°C and was eluted from the erythrocyte-virus complex on subsequent incubation at 37°C. On the other hand, the Rauscher virus did not agglutinate and was not significantly adsorbed by the erythrocytes, judging by the opalescence characteristics of the density gradient. The incubated viral mixture agglutinated the erythrocytes, and the density-gradient examination showed that the erythrocytes had absorbed the interviral product, leaving only a minor opalescence corresponding to the excess of Rauscher virus normally present in the incubated viral mixture. Thus most of the Rauscher virus component in the mixture was adsorbed, whereas the



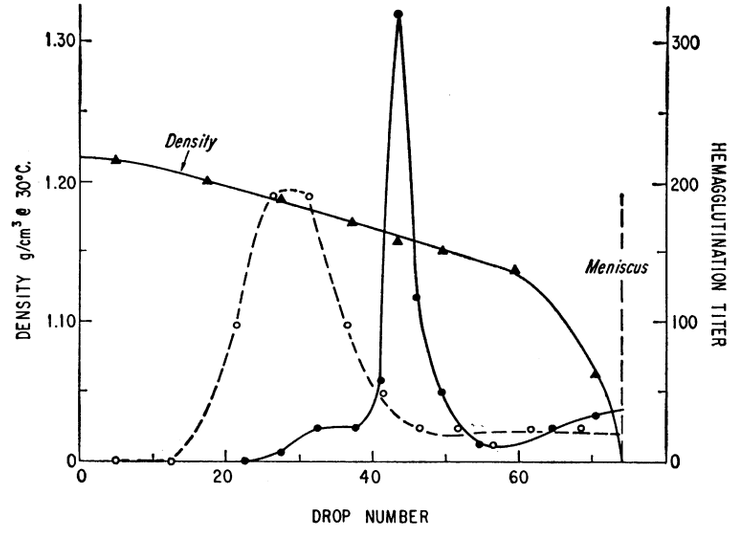


Fig. 1 (left). Density gradient centrifugation of incubated viral mixture and of separate viruses; the three tubes contained identical preformed potassium citrate gradients (4.5 ml, density 1.13 to 1.26 g/cm³ at 30°C). Portions (0.5 ml) of the separate viruses or of the incubated mixture were layered on the gradients. The tubes were then simultaneously centrifuged for 2 hours at 36,000 rev/min on the SW39L rotor at 4°C in the Spinco preparative ultracentrifuge. *Left tube*, Rauscher virus; *center tube*, incubated mixture of equal volumes of Rauscher and influenza viruses; *right tube*, influenza virus. Fig. 2 (right). Distribution of hemagglutinin on potassium citrate gradients after simultaneous centrifugation of portions (0.5 ml) of influenza virus alone (○—○) and the incubated equal-volume mixture of influenza and Rauscher viruses (●—●) on separate but identical potassium citrate gradients (4.5 ml, density 1.115 to 1.225 g/cm³). The tubes were centrifuged for 1.25 hours at 36,000 rev/min on the SW39L rotor at 4°C. Under these conditions the materials on the gradients approached but did not attain equilibrium. The tubes were punctured, and multidrop fractions were collected and assayed for hemagglutinin.

Rauscher virus alone was not adsorbed. This experiment demonstrated that the interviral product really formed during incubation of the viral mixture and prior to the density gradient centrifugation. The interviral product was partially eluted when the erythrocyte–interviral product complex was incubated for 3 hours in PBS at 37°C (*3*).

We have considered the possibility that the interviral product obtained by incubating a mixture of the two viruses may be an interviral aggregate. Homopolymer viral aggregates have been shown to form readily in concentrated solutions of the more simply structured nucleoprotein viruses (*4*).

While the following evidence does not definitely exclude an interviral aggregate, it does appear to be in poor accord with such a structure. Each virus alone shows considerable heterogeneity of density on shallow potassium citrate gradients. Interviral aggregates of two such heterogeneous populations of particles might be expected to yield a population of aggregates that would also show comparable heterogeneity. Yet the interviral product shows a very narrow density distribution on an identical gradient. Retention of complete egg infectivity and hemagglutinin by the influenza component in the interviral product hardly accords with an aggregate structure—unless it is simultaneously postulated that the aggregate dissociates to its components in the dilutions attendant on bioassays—since aggregation might be expected to mask the cell attachment and hemagglutinin sites on the viral surface. An interviral aggregate might be expected to sediment more rapidly on centrifugation than either of its constituents alone. A centrifugation experiment in which the separate viruses and the incubated mixture were simultaneously centrifuged (18,000*g*) on identical but separate shallow potassium citrate gradients (density 1.04 to 1.13 g/cm^3) indicated that the interviral product had a sedimentation velocity intermediate between those of the single viruses, but quite close to that of the more rapidly sedimenting influenza virus. Preliminary electron microscopic studies (*9*) indicated that the interviral product as isolated from the potassium citrate gradient contained large numbers of virus particles which showed no appreciable clumping or aggregation. During the characterization on the citrate gradient the interviral product resisted forces of over 70,000*g* in a medium of over 1*M* potassium citrate concentration. Furthermore incubation of the Rauscher virus with receptor destroying enzyme (RDE, 30 units per milliliter of virus) before incubation with the influenza virus in the presence of the enzyme did not appreciably inhibit formation of the interviral product. The interviral product was also as readily obtained when the viruses were mixed and incubated at 37°C, and the subsequent characterization on the potassium citrate gradient was effected at 30°C.

Table 1. Comparison of biological properties of an incubated mixture of the Rauscher virus (RV) and influenza virus (IV) with those of the individual viruses.

Mixture	Hemagglutination titer*	Egg infectivity ID_{50}	Mouse leukemogenic potency†			
			Splenomegaly (No.)	Time to palpable splenomegaly (av. in days)	Deaths (No.)	Time to 50% mortality (days)
0.33 ml RV + 0.66 ml IV	512	8.5	14/14	16	13/14	40
0.33 ml RV + 0.66 ml 0.05 *M* citrate buffer			10/10	12	9/10	40
0.66 ml IV + 0.33 ml 0.5*M* citrate buffer	512	8.2				

* Serial dilutions (twofold) in physiological saline were incubated with 1 percent by volume of chicken erythrocytes at room temperature. † Sample diluted to 4 ml in PBS (*p*H 7) containing 1 percent of heat-inactivated calf serum; 72-hour-old Balb/c mice were then inoculated with 0.1 ml of the dilution.

The rather poor fit of the data with an interviral aggregate structure compels us, by a process of elimination, to weigh an alternative explanation. We consider the possibility that the Rauscher and influenza viruses may be effectively fused by mutual coalescence of their limiting membranes on contact. Solubility and surface tension forces may then lead to rearrangement of the structural constituents of both viruses, and disruption of the temporarily fused state may lead to the finally observed set of particles, among which the original genomes and other components of both viruses are distributed. Existing evidence shows that considerable variation in size, density (*5*), and hemagglutinin and hemolysin content (*6*) is consistent with infectivity for individual particles within a single species of membrane-containing viruses. Since viral progeny obtained from mixed infections by different strains of either influenza viruses or Newcastle disease viruses can contain heterozygotic infectious particles (*7*), it is possible that myxoviruses may possess some latitude in the amount of nucleic acid they may contain. Hoyle has published substantial evidence that influenza virus can physically interact with cytoplasmic particles, obtained by incubation of normal chicken chorioallantoic membrane, in such a manner that the limiting membranes of the virus and the cytoplasmic particles are considerably exchanged without significant change in either the hemagglutinin or infectivity titers of the virus (*8*). In that instance, however, prior treatment of the cytoplasmic particles with RDE was reported to prevent the reaction. The scheme we are considering here is generally similar to that deduced by Hoyle, except that we are considering the possibility that the influenza virus may react with another virus rather than with a cytoplasmic particle. This hypothesis is apparently consistent with the available data, but as yet we have no direct proof of its validity.

By the density-gradient procedure we examined the behavior of other pairs of membrane-limited viruses in vitro to determine the generality of interviral reactions and their possible relevance to disease processes. The results appear to provide examples of both the occurrence and failure of interviral reactions. Thus the formation of an interviral product involves some specificity in the reactants, so that the relevance of the interviral phenomenon will require careful analysis in each biological situation. Viral interactions may well not be limited to viruses with different densities; other subtle physical and biological techniques may be useful in detecting interviral products.

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same as that of the interviral product before hemadsorption, except that in some experiments it showed a shift toward the density of the influenza virus. The photographic records of our experiments indicate that this shift in density occurred when the incubated viral mixture contained some excess influenza virus which was hemadsorbed along with the interviral product. We therefore consider that the original interviral product and the excess influenza virus may have further reacted after elution to yield a final product of higher density.

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Spalled, Aerodynamically Modified Moldavite from Slavice, Moravia, Czechoslovakia

Abstract. *A Czechoslovakian tektite or moldavite shows clear, indirect evidence of aerodynamic ablation. This large tektite has the shape of a teardrop, with a strongly convex, deeply corroded, but clearly identifiable front and a plano-convex, relatively smooth, posterior surface. In spite of much erosion and corrosion, demarcation of the posterior and the anterior part of the specimen (the keel) is clearly preserved locally. This specimen provides the first tangible evidence that moldavites entered the atmosphere cold, probably at a velocity exceeding 5 kilometers per second; the result was selective heating of the anterior face and perhaps ablation during the second melting. This provides evidence of the extraterrestial origin of moldavites.*

In Bohemia and Moravia during March and April of 1964, I inspected over 15,000 moldavite specimens. My main purpose was to uncover moldavites showing evidence of aerodynamic ablation or containing various inclusions. Most specimens are in the National Museum in Prague; many are in the private collections of Jan Oswald of Český Budějovice (3500) and Josif Prokopec of Český Krumlov (500). A few of the moldavites inspected belong to Charles University and other private collectors. Most of the specimens are fragments, mostly platy and some subrounded. Many are of teardrop shape. A few are rod- or disc-shaped; a few are curved plates. Dumbbell-shaped and core-shaped moldavites are rare, but do occur. A striking feature of the assortment and frequency of shapes of the moldavites is their similarity to those of Thailand and Indochina.

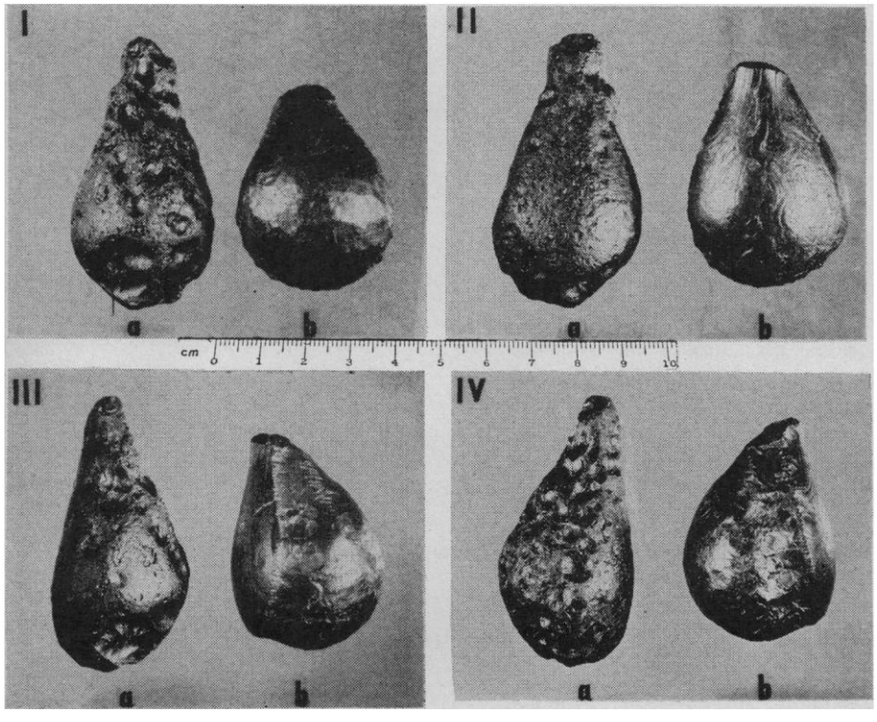

Fig. 1. Comparison of a spalled moldavite teardrop from Slavice (*a*) with a spalled australite teardrop from Renmark, South Australia (*b*). I, Anterior view; II, posterior view; III, side view, with posterior side to the left; IV, side view, with posterior to the right.

Of the many specimens inspected, the specimen described herein is the only one showing indirect evidence of aerodynamic ablation. It is a large, teardrop-shaped moldavite collected by Jaromir Šofr of Třebíč from a plowed field in an area of moldavite-bearing gravel adjoining the west side of the village of Slavice in Moravia. It is greenish brown in color and translucent. It is 67 mm long, 33 mm wide, and 30 mm thick at the maximum cross section, perpendicular to the long axis; it weighs 64.75 g. Except near the tip of the teardrop which is slightly chipped in two places, the gross teardrop shape is well preserved.

The profile is best observed with the specimen lying on its side (Fig. 1, *a* in parts III and IV). The highly convex anterior side (Fig. 1, *a* in parts I, III, and IV) is covered with deep or shallow pits of corrosion origin. The local flow structure or schlieren is revealed in and across the pits. The plano-convex posterior side is relatively smooth and well preserved (Fig. 1, *a* in part II), in spite of local crescent-shaped percussion marks indicating erosion during transport in running water. The break in the curvature between the plano-convex posterior and the strongly convex anterior portion (the keel) is evident and locally well preserved (Fig. 1, *a* in part III); elsewhere the keel is not so sharply defined.

An ablated and spalled large australite teardrop is shown for comparison (Fig. 1*b*). With australites, flaked cores, boats, dumbbells, and flanged buttons show various stages of spalling as represented by specimens which partially retain the ablated front and side surfaces; these are known as "indicators" (*1*, *2*). Figure 2*a* shows an indicator of an australite button with most of the anterior portion spalled off. The ring waves on the remnant unspalled piece make it clear that spalling of the aerothermal stress shell did