

Fig. 1. Huntite flowstone that has become desiccated, cracked, and subsequently curled along its cracked edges. [Photographed by Pete Lindsley, Dallas, Texas]

ered as a wall flowstone in Carlsbad Caverns, New Mexico. This flowstone is located in a small alcove about 40 m down a side passage continuous with the Lunchroom (8). It appears to be forming actively along with a number of tubular stalactites, helictites, and draperies. Moonmilk, which is usually prevalent in the lower portions of Carlsbad Caverns, is noticeably absent along the entire side passage. The huntite flowstone occurs as a chalk-white, microcrystalline layer (1 mm thick) that appears to be buckled away from a more coarsely crystalline flowstone of a different composition. The huntite layer has become desiccated and cracked in places. The edges of the cracked layer have curled so that the total display resembles a mass of cornflakes or Chinese fortune cookies (Fig. 1). The huntite flowstone is approximately 3 m in vertical extent and a maximum of 1 m in width. Individual crystals of huntite measured from 1 to 57  $\mu\text{m}$ , with the most common size in the range of 10 to 20  $\mu\text{m}$ . The huntite was identified by x-ray powder patterns, as were five other minerals in the vicinity. Specifically, the wall bedrock is calcite; some fine powder on the floor approximately underneath the huntite flowstone is also calcite; the more coarsely crystalline flowstone underlying the crinkled huntite layer is dolomite; a light tan flowstone on the opposite wall of the

alcove (about 2 m from the huntite flowstone) is calcite; narrow chalk-white ribbons of flowstone overlying the tan calcite flowstone are aragonite.

These carbonate minerals appear to be precipitating directly from solu-

tions containing varying amounts of calcium and magnesium. Minerals which have precipitated from solutions with larger  $\text{Ca}^{2+}/\text{Mg}^{2+}$  ratios are overlain by a mineral which has precipitated from solutions with lower  $\text{Ca}^{2+}/\text{Mg}^{2+}$  ratios, namely, huntite over dolomite or aragonite over calcite (5). The absence of hydromagnesite moonmilk suggests that the huntite and the dolomite are not alteration products of hydromagnesite; however, the dolomite may have been altered from the huntite, as suggested by Moore (4).

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#### References and Notes

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9. Investigations were made in the undeveloped portions of Carlsbad Caverns and photographs were taken there with the permission and cooperation of the National Park Service. Field support was received from the Guadalupe Cave Survey.

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## Suspensions: Fluids with Fading Memories

**Abstract.** *A sheared liquid suspension whose flow is reversed remembers with various degrees of perfection all earlier configurations of the particles. The memory effects, studied primarily because of their importance in suspension rheology, may be of wider significance.*

When a suspension of particles in a liquid undergoes a simple shear, each particle translates and rotates and interacts with others. Under proper conditions (1), the translational and rotational movements are retraced exactly when the direction of the motion of the fluid is reversed so that every pre-existing configuration of the particle assembly is restored: the suspension thus possesses perfect memory (2). Changing the conditions (3) reduces the reversibility and impairs the memory by measurable amounts. We report the phenomena because of their importance in suspension rheology (4) and their potential use as models of information storage, memory, and time reversal (5) systems which are amenable to experimental and computational manipulation (6).

In our experiments we have used particles large enough to photograph, generally with a cine camera attached to a microscope, so that we can measure as many as three rotational and three translational coordinates of each of a number of particles at various times. Thus, if we take  $n$  pictures of  $N_0$  particles, we can describe all particle configurations by  $6nN_0$  coordinates, a number which can easily be made sufficiently large to have statistical significance.

As an example, we consider a dilute suspension (up to 100 particles per milliliter) of cylindrical rods, microtomed to a length of 870  $\mu\text{m}$  from metallized nylon monofilaments 175  $\mu\text{m}$  in diameter, suspended in an oil of the same density, and placed in the annulus between two vertical concen-

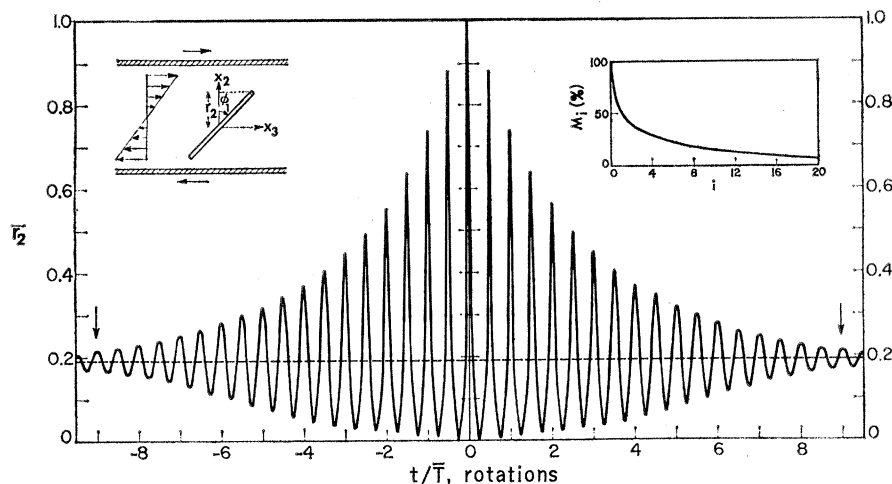


Fig. 1. Calculated changes in the mean projections  $\bar{r}_2$  resulting from the spread in axis ratios of the rods ( $\bar{r}_0 = 10$  and  $\sigma = 0.2$ ), showing damped oscillations in a system with perfect memory (solid line). The horizontal dashed line is the equilibrium value in a system which has been perturbed to destroy the memory ( $M_\infty = 0$ ). (Left inset) Coordinate system, with the  $X_1$ -axis normal to the plane of the figure. (Right inset) Decay in the memory  $M_i$  of a perturbed system after  $i$  reversals for perturbations at  $t/\bar{T} = \pm 9$  (shown by arrows in the main part of the figure) when all the particles of a particular  $\phi_1$  were given disturbances in  $\phi_1$  by identical bursts of rotary diffusion (7).

tric cylinders. With the cylinders at rest, we applied an electric field (60 hertz,  $0.5 \text{ kv cm}^{-1}$ ) between the cylinders, thus aligning the rods parallel to the  $X_2$ -axis (Fig. 1, left inset). After the electric field was removed, the suspension was sheared at  $\pm 0.5 \text{ sec}^{-1}$  by rotating one or both of the cylinders so that each of the six coordinates of every particle changed reversibly.

We have predicted that various statistical properties of the suspension such as the distribution of particle orientations, and related rheological properties such as the viscosity and the normal stress differences, will undergo reversible oscillations, usually damped (4). A convenient measure of the distribution of particle orientations, involving only two rotational coordinates, is the mean projection of the unit length  $\bar{r}_2$  of the rods along the  $X_2$ -axis (Fig. 1, left inset).

By satisfying the conditions in (1) we have shown experimentally that  $\bar{r}_2$ , averaged over about 100 particles, exhibited damped reversible oscillations of frequency  $2/\bar{T}$ , where  $\bar{T}$  is the mean period of oscillation of a single isolated rod about the  $X_1$ -axis (the optic axis for photography), in accordance with the theoretical scheme shown in Fig. 1. The relaxation time  $\tau$  during which the amplitude of oscillation decayed to  $1/e$  of the initial amplitude agreed with the approximate theoretical relation (4)

$$\bar{T}/\tau = b_1 + b_2 N \quad (1)$$

where  $b_1$  and  $b_2$  are calculable constants and  $N$  is the number of particles per milliliter;  $b_1$  is an inherent rate of damping due to the spread in axis ratios of the particles, which we express as the standard deviation  $\sigma$  from the mean equivalent ellipsoidal axis ratio  $\bar{r}_0$  of the rods, and  $b_2 N$  is a rate of damping resulting from two-body interactions.

We have also shown experimentally that it is easy to impair the memory of the system (6). We demonstrate the effect, however, by recourse to the theory for the case  $N \rightarrow 0$ , for which exact calculations can be made when  $\bar{r}_0$  and  $\sigma$  are known. Under reversible conditions  $\bar{r}_2$  undergoes the damped oscillations shown in Fig. 1. On reversal of the flow at any time, the oscillations can be built up reversibly until zero time is regained when, in negative time, they decay as mirror images of those in positive time. The pattern can, in principle, be repeated indefinitely by cycling back and forth in time.

A simple computational method of impairing the memory is to stop the motion at a particular time, say  $t/\bar{T} = 9$ , perturb the angular displacements  $\phi_1$  (Fig. 1, inset) of all the particles according to a randomizing scheme (7), reverse the motion until  $t/\bar{T} = -9$ , and then repeat the process. The oscillations in  $\bar{r}_2$  are no longer exactly restored following each reversal, as we can readily show from the progressive

decrease in  $\bar{r}_2$  at zero time after  $i$  reversals. We can express the memory of the system in a number of ways, a simple one being

$$M_i = \frac{\bar{r}_{2i} - \bar{r}_{2e}}{\bar{r}_{20} - \bar{r}_{2e}} \quad (2)$$

As shown in Fig. 1 (right inset),  $M_i$  decays from 100 percent (perfect memory) to zero at  $i = \infty$  when  $\bar{r}_2$  (shown as the dashed line in Fig. 1) is flat, and the memory of the initial imprint is completely lost. We have shown by calculation that the farther we go from time zero before perturbing the system, the greater is the memory impairment from one reversal to the next, in general accord with our experimental findings. We predict corresponding behavior in the intrinsic viscosity and normal stress differences, striking manifestations of non-Newtonian behavior which persist until  $i = \infty$  when the system should be Newtonian (6).

We have illustrated the scheme with an example which is experimentally realizable and theoretically tractable. By changing the shape, size, deformability, and concentration of the particles, the type of fluid, the duration of each cycle of shear, the number of flow reversals, and the type of perturbation—some of which changes have been made experimentally (3, 6)—a wide range of memory patterns can be achieved.

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#### References and Notes

1. The general requirements for reversibility are that the linearized form of the Navier-Stokes equation applies and that the boundary conditions are identical for forward and reverse flows. The specific requirements are that relative to the time scale of the observations (6): (i) the Reynolds numbers are zero and the shear flow is geometrically perfect; (ii) the fluid is Newtonian; and (iii) the particles are rigid, nonsedimenting, electrically neutral, large enough for Brownian motion and van der Waals attraction to be negligible, and free of external forces and couples. See W. Bartok and S. G. Mason, *J. Colloid Sci.* **12**, 243 (1957); F. P. Bretherton, *J. Fluid Mech.* **14**, 284 (1962); J. C. Slatery, *ibid.* **19**, 625 (1964); E. Anczurowski, R. G. Cox, S. G. Mason, *J. Colloid Interface Sci.* **23**, 547 (1967).
2. The disappearance of dyed letters when a liquid is sheared, followed by their reappearance when the shear is reversed, provides a vivid and simple demonstration of the effect. Because of diffusion of the dye the restoration of the letters is generally not perfect. See J. P. Heller, *Amer. J. Phys.* **28**, 348 (1960); H. L. Goldsmith and S. G. Mason, in *Rheology: Theory and Applications*, F. R. Eirich, Ed. (Academic Press, New York, 1967), vol. 4, p. 85.
3. Incomplete reversibility is caused by failing to meet one or more of the requirements listed in (1), for example, by increasing the Reynolds number so that inertial effects be-

- come appreciable, permitting temperature gradients with resulting thermal convection, using small or electrically charged particles so that diffusion or interparticle attraction can occur, employing non-Newtonian fluids, applying an external electric field, and so forth. See R. S. Allan and S. G. Mason, *Proc. Roy. Soc. Ser. A* **267**, 62 (1962); A. Karnis and S. G. Mason, *Trans. Soc. Rheol.* **10**, 571 (1966); F. Gauthier, H. L. Goldsmith, S. G. Mason, *Rheol. Acta* **10**, 344 (1971); E. B. Vadas, H. L. Goldsmith, S. G. Mason, *J. Colloid Interface Sci.*, in press.
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7. Many modes of perturbation can be considered. A simple one is to perturb all particles having identical  $\phi_1$  at a particular  $t/T$  ( $\pm 9$  in the example) in the direction of the equilibrium distribution of orientations for each value of  $\phi_1$ , by using a Gaussian distribution of perturbations of the phase angles  $\kappa$  given by  $\tan \phi_1 = r_0(\tan 2\pi t/T + \kappa)$ . This corresponds to identical bursts of rotary diffusion at each reversal. The values of  $M_2$  in Fig. 1 are calculated (6) for perturbations of  $\kappa$  with a standard deviation of 0.2 radian at each reversal.
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## Antibody to HSV-2 Induced Tumor Specific Antigens in Serums from Patients with Cervical Carcinoma

**Abstract.** Antibody distinct from that involved in neutralization and directed to an antigen (AG-4) induced in HEp-2 cells by infection with herpesvirus type 2 was identified in serums from patients with cervical carcinoma by means of a quantitative micro complement fixation test. The presence of antibody to AG-4 correlates well with the extent of the tumor; antibody is virtually absent in matched control women and in women with therapy and without recurrent neoplasia. Reactivity is not observed with control antigen consisting of a cell extract prepared from uninfected HEp-2 cells. The possible prognostic significance of this antibody and its implications are discussed.

Herpesvirus type 2 (HSV-2), a virus biologically and antigenically distinct (1) from that associated with facial lesions, was isolated from cervical lesions (2) and male genitourinary samples (3) and was shown to be venereally transmitted (4). A relation between HSV-2 and squamous carcinoma of the human cervix has been suggested on the basis of seroepidemiologic studies, indicating a significantly higher prevalence of HSV-2 antibody in patients with both invasive (5) and preinvasive (6, 7) cervical neoplasia as compared to controls. A similar association was not noted with other venereal diseases (7).

In addition to the seroepidemiologic evidence linking HSV-2 and cervical neoplasia, evidence of virus persistence in cervical tumor cells is based on three observations, as follows. (i) Exfoliated tumor cells contain viral antigens (8, 9), (ii) a type 2 herpesvirus was isolated from cervical neoplastic

cells grown in culture (10), and (iii) DNA sequences corresponding to part of the HSV-2 genome were found in a cervical tumor (11). Finally Duff and Rapp (12) reported the induction of tumors in hamsters inoculated with hamster cells transformed by ultraviolet inactivated HSV-2.

In order to determine whether virus specific products are expressed in cervical tumor cells, we attempted to find, in serums from patients with cervical carcinoma, antibody to HSV-2 antigens other than those involved in neutralization. Such a finding would be analogous to the observation that tumors and cells transformed by SV40 and polyoma virus contain a specific antigen (T) which is distinct from that of the virus particles and which reacts with serums of tumor-bearing hamsters (13, 14). As reported here, infection of HEp-2 (human epidermoid carcinoma) cells with HSV-2 for 4 hours leads to the production in these cells of antigen

or antigens (AG-4) detected by complement fixation with human serums from patients with cervical neoplasia but not with serums from female control subjects, even though these serums may have equally high levels of antibody to HSV-2, when tested by neutralization.

Serums were obtained from 84 patients diagnosed as atypia (20 patients), carcinoma in situ (19 patients), and invasive cervical carcinoma (45 patients), and from 84 control subjects (from age 20 to 80 years) admitted to the Johns Hopkins Hospital for illness unrelated to cervical cancer and having a negative history of malignancy. These subjects were matched to the carcinoma patients for age, race, sex, and socioeconomic class according to economic deciles of the resident census tracts for the City of Baltimore. Of the invasive cancer patients, 22 women were untreated, and 23 had therapy 2 months to 19 years (mean, 4 years) prior to blood collection. Only one of these (No. 60) had histologic evidence of recurrent neoplastic disease. A third group consisted of women with malignancies at sites other than the cervix. At the time of blood collection, all subjects were free of active genital herpetic infection as determined by Papanicolaou smears and pelvic examination (15).

HEp-2 cells were infected with 0.2 to 0.4 plaque-forming units of HSV-2 (16) per cell. After adsorption at 37°C, for 1 hour, the cells were overlaid with maintenance medium consisting of medium 199 supplemented with 1 percent fetal calf serum (Grand Island) and re-incubated at 37°C for 4 hours. At this time, the cells were collected by scraping, washed with barbitol-buffered saline at pH 7.4 (17), and disrupted by freezing and thawing. Cell debris was removed by centrifugation at 1550g for 30 minutes, and the supernatant, designated AG-4, was used in complement fixation tests. A crude extract prepared in the same way from HEp-2 cells treated only with maintenance medium

Table 1. The occurrence of antibody to AG-4, AG-H, HSV-2, and HSV-1 in patients with untreated and treated invasive carcinoma of the cervix.

Group	Tested (No.)	Mean age	Mean economic decile	Positive for anti-AG-4		Positive for anti-AG-H		Positive for anti-HSV-2		Positive for anti-HSV-1	
				No.	%	No.	%	No.	%	No.	%
Invasive cancer	22	52	4.4	20	91	0	0	22	100	18	82
Matched controls	22	50	4.7	2	9	5	23	15	68	19	86
Invasive cancer with Radiation	14	53	3.8	0	0	1	7	14	100	13	92
Hysterectomy	9	46	4.7	0	0	0	0	9	100	6	67
Total treated	23	50	4.3	0	0	1	4	23	100	19	82