write you at least once during a visit to Sweden. Now again I am here, and this time I shall have to stay until Denmark is liberated. About the middle of June I had an intimation of imminent danger and had to go "underground," which in my case meant going about in North Zealand on my bicycle and staying for periods not too long with various friends and relatives. I am sorry to say that I had not really deserved this honour, having done very little towards the cause, and I fear it was only because I was considered "prominent" that I might be worth "liquidating." I had arranged to spend the summer at a small and secluded limnological laboratory, when I received word from Stockholm that my son-in-law, Christer Wernstadt, was very seriously ill, and could I come. After deliberation with well-informed persons it was decided that I should apply for regular visas and try to come "legally" to Sweden, and this succeeded in such an incredibly short time that there is a strong suspicion that the German official in question knew of the danger and wished to be helpful. Anyway I arrived safely on July 9th.

In addition to the Nobel Prize, August Krogh received honorary degrees and memberships in scientific societies from many parts of the world. It is our pleasure that he was one of those given the doctorate at the Harvard Tercentenary in 1936, and thus, in addition to having had a hand in the training of eight full professors at Harvard, he appears upon the rolls of the university.

Those who knew him will never forget this man and those not so fortunate will ever gain inspiration from his clear-sighted and classically simple observations.

Technical Papers

A Limitation on the Ultracentrifuge Separation-Cell Technique[1]

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The ultracentrifuge separation cell has been increasingly utilized in recent years in investigations of the biological activity associated with certain high molecular weight solutes (*2*, *3*, *8*). In this analytical cell a porous plate overlaid with filter paper divides the cell into two compartments. This permits occurrence of analytical sedimentation, observed by the usual optical methods, but prevents remixing of the contents of the two chambers at the end of an experiment. The sedimenting boundary of the characteristic substance suspected of being biologically active may then be brought to various known positions in the cell in different experiments, the rotor rapidly decelerated, and the biological activity remaining in the top compartment of the cell determined. If one finds that the residual activity is exactly proportional to the amount of the characteristic substance remaining in the top chamber, one has apparently eliminated the possibility that impurities of either significantly smaller or significantly larger sedimentation rates than that of the characteristic substance are the active principles.

[1] Presented before the American Chemical Society at the meeting in Philadelphia, April 9–13, 1950.

[2] Postdoctoral research fellow, National Institutes of Health, United States Public Health Service.

[3] Contribution No. 1427.

It is not clear, however, that this conclusion is justifiable under the following set of circumstances. Let us suppose that we have a solution containing a characteristic substance (hereinafter referred to as CS) in relatively high concentration (1–10 mg/ml), together with a very small amount of an active principle (AP), which is responsible for all of the particular biological activity in this mixture. Let us further assume that the sedimentation constant of the AP is larger than that of the CS. Upon performing a separation-cell experiment with this mixture, might not the small thermal gradients occurring during sedimentation, or the vibration of the rotor, be sufficient to disrupt the AP sedimenting boundary, since the density gradient across this boundary would be very small? Furthermore, might not the AP then be distributed relatively uniformly ahead of the stable CS boundary, but not be convectively transported across it? Under these circumstances, the gradient of activity through the cell might very nearly coincide with the gradient of concentration of the CS, creating the illusion that the CS is the active principle.

In order to examine this problem, which is of considerable interest, for example, in the study of plant viruses (*2*), we must know more about the behavior of very dilute solutions in the ultracentrifuge. The usual optical methods for determining sedimentation rates are not sensitive enough to permit the study of solutions of concentration lower than about 0.1 mg/ml. Bacteriophage particles, however, offer the possibility of studying this problem. Their concentrations can be followed accurately by infectivity measurements in extremely dilute solutions. Despite the fact that some of these bacteriophages have a morphology which makes the term "molecules" inapplicable to them, they behave like protein molecules in the usual sedimentation and diffusion

experiments. Sharp *et al.* (*7*) investigated relatively concentrated T2 *Escherichia coli* bacteriophage solutions in the ultracentrifuge by the ultraviolet absorption optical method. Polson (*4*) studied the diffusion behavior of T4 and T3 bacteriophages, and showed that the tail of the former particle does not significantly affect its diffusion properties. Both particles obey the Einstein equation for diffusion reasonably well in relatively concentrated solutions. A review of the molecular kinetic properties of bacteriophages was recently published in this journal (*6*).

Our experiments were performed with T2 bacteriophage. The raw bacterial lysates, which were prepared in M-9 synthetic medium, were concentrated and purified by differential centrifugation. The infectivity of the resulting preparation was of the order of $10^{-15.9}$ g N/infective particle.

The ultracentrifuge used in these studies was designed and built in the Chemistry Department of this institute. A detailed description of it will appear elsewhere.

Various concentrations of bacteriophage in cacodylate buffer, pH = 6.9, $\mu = 0.1$, were examined by the separation-cell technique. In experiment U-46, with a solution containing 0.5 mg/ml, the sedimentation was followed by the usual Philpot Schlieren optical method. Two peaks were recorded with sedimentation constants of about 1,000 and 700 *S*. This two-peak phenomenon was observed by Sharp *et al.* (*7*). After 2,300 sec of sedimentation at 149.3 rps the slower peak was well past the porous plate partition. All the experiments, the results of which are given in Table 1, were run for the same time at the same speed.

TABLE 1

Exp. No.	Original solution		Solution removed from top chamber	Residual activity %
	Titer T2/ml	Conc. mg/ml	Titer T2/ml	
U-46	6.0×10^{11}	5.0×10^{-1}	4.4×10^{9}	0.7
U-45d	1.1×10^{10}	9.1×10^{-3}	1.8×10^{8}	1.6
U-45c	8.0×10^{8}	6.6×10^{-4}	5.1×10^{7}	6.4
U-45b	6.1×10^{7}	5.1×10^{-5}	2.2×10^{6}	3.6
U-45a	6.5×10^{5}	5.4×10^{-7}	2.8×10^{4}	4.3

A calibrated thermocouple with one junction situated in the slip stream of the rotor indicated that the temperature of the rotor changed by no more than 0.2° during the runs. An air pressure of 5×10^{-2} mm Hg was maintained in the centrifuge chamber and no cooling of the chamber was necessary at this low speed. Control experiments indicated that the bacteriophage suffered no change in infectivity upon standing at room temperature in the separation cell for comparable times.

These experiments indicate that an increasing proportion of the bacteriophage particles is left in the top compartment upon sedimenting more dilute solutions under identical conditions. This could not be due to a displacement of the mean ordinate of the sedimenting boundary since, in the absence of disturbing influences, the sedimentation constant should increase with dilution (*1*). The results suggest rather that convection currents increasingly perturb the concentration distribution about the mean ordinate of the boundary with increasing dilution.[4] There does not appear, however, to be a concentration at which an abrupt change takes place in the stability of the boundary.

TABLE 2

Exp. No.	TMVP Boundary position (see text)	Bacteriophage titers $\times 10^{-6}$		Residual activity %
		original solution	residual solution	
U-5a	0.50	3.8	0.15	4.0
U-7	0.88	2.1	0.033	1.6
U-6	1.25	2.1	0.024	1.1

A second set of experiments was performed with mixtures of 5 mg/ml of tobacco mosaic virus protein (TMVP) and about 10^6 T2 bacteriophage/ml in the cacodylate buffer used previously. The TMVP boundary, moving at a rate equivalent to about 200 *S*, was sedimented to various positions in the cell. In experiment U-5a, the boundary was taken 0.50 of the distance from the top of the cell to the porous plate partition; in experiment U-7, 0.88 of this distance; and in experiment U-6, about 0.25 of this distance *past* the partition. The contents of the top compartment were then analyzed for bacteriophage activity. Control experiments indicated that TMVP has no effect on bacteriophage infectivity.

The results of these experiments are listed in Table 2. The fact that the titers in experiments U-7 and U-6 of the solutions removed from the top compartment were only slightly different demonstrates that no sharp change in bacteriophage concentration occurs across the slower moving TMVP boundary. In other words, the convective transport of the bacteriophage particles is not seriously affected by the presence of the stable, slower moving boundary.

If the results obtained in these experiments are of general validity, we may summarize them as follows:

a) Thermal gradients and other perturbing influences such as vibration occurring during analytical ultracentrifugation are apparently capable of disturbing but not completely disrupting the sedimenting boundaries of very dilute solutions.

b) The convective transport of the dilute solute is not significantly affected by the presence of a stable, slower moving sedimenting boundary.

c) It should therefore be feasible to eliminate in the following manner the possibility that a certain biological activity is carried by an impurity with a higher sedimentation constant than that of the CS. The CS boundary may be sedimented about 0.5 of the distance to

[4] The large increase in the apparent diffusion rates of bacteriophage particles in the concentration range from 10^9 to 10^5 particles/ml, observed by Polson and Sheppard (*5*), may also be interpreted as due to an increased convective transport of the solute in more dilute solutions.

the partition in the separation cell, and the contents of the top compartment analyzed for activity. These contents may then be diluted to a volume sufficient to refill the cell and the above experiment repeated. After several such cycles, if the ratio of activity to the amount of the CS left in the top compartment remains constant, then it may be concluded that no substance, whatever its concentration, sedimenting significantly faster than the CS, is active.

Similar considerations should apply in electrophoresis separation-cell experiments.

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Germaniferous Lignite from the District of Columbia and Vicinity[1]

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A unique accumulation of germanium has recently been found in lignite remains of *Cupressinoxylon wardi* Knowlton, 1889, from the Patuxent formation (Lower Cretaceous) in the District of Columbia and vicinity. The discovery was made in the course of spectrographic studies on concentrations of germanium, gallium, vanadium, and other elements in ash of American coals.

The highest content of germanium heretofore reported was in germanite from Tsumeb, Southwest Africa, and from the Belgian Congo; that mineral contained 6–10 percent. The ash of *C. wardi* contains up to 6 percent and many of the samples contain 3–5 percent. The ash content of the samples (air-dry basis) is between 2 and 9 percent. The average content of germanium in the crust of the earth is estimated to be 1×10^{-4} percent. Consequently, in the ash of *C. wardi* the concentration is more than 10,000 times the average.

The ashes of samples of *C. wardi* also contain vanadium (0.7%–5.0%), chromium (0.1%–0.8%), and gallium (0.03%–0.2%). Some of the samples show a large concentration of copper. Examination of the ash of Pleistocene wood in this area indicates contents of a few hundredths of 1 percent of germanium.

The *C. wardi* was identified by R. W. Brown of the U. S. Geological Survey. We are also indebted to Henry Mela, Jr., and F. S. Grimaldi of the Trace Elements Section of the Survey for confirmatory chemical analyses for germanium.

[1] Published by the permission of the director, U. S. Geological Survey.

Rapid Carbon Dioxide Test for Sickling

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Sickle cell disease is a notorious mimic and deceiver. There is need for a method to demonstrate sickling which is rapid, dependable, and simple. Sickling tests in present use are not completely satisfactory. The sealed moist preparation method (*5*), the moist stasis test (*9*), and the oil-sealed tube technique (*1*) may not give positive results for many hours. The rapid bacteriologic method (*11*) requires caring for the perpetuation of the culture and does not lend itself to use in the physician's office. Recently advocated methods involving the use of various reducing substances such as BAL (*12*), cysteine (*12*), hydrogen sulfide (*12*), sodium dithionate (*8*), sodium hydrosulfite (*4*), cevalin (*3*), and sodium bisulfite and ascorbic acid (*3*) share the common disadvantage that these reducing agents are extremely unstable and must be freshly prepared.

We wish, therefore, to describe a simple and rapid method, utilizing carbon dioxide, by which sickling can be regularly demonstrated within 5 min from the time the blood sample is drawn. The test can be conveniently performed in the physician's office.

Five to 10 ml of venous blood is collected in an oxalated tube. The blood is transferred to a 250-ml Erlenmeyer flask, and a stream of pure carbon dioxide from a small, commercially available cylinder is directed into the neck of the flask for 10–15 sec. The flask is then immediately stoppered, and the blood is gently swirled about several times. The blood darkens. After the flask has remained stoppered for 5 min, the cork is removed and a drop of blood is quickly transferred by means of a pipette to a clean cover glass. A vaseline-sealed preparation is immediately made on a slide, and the cells are viewed under high-dry magnification. Speed is essential in the transfer of the drop of blood from the flask and in making the vaseline-rimmed preparation. The presence of unequivocal sickling under high-dry magnification indicates a positive result.

Twenty-seven Negro patients who showed delayed sickling after 1–57 hr on the routine sealed preparations (*5, 9*) all gave positive findings immediately with the carbon dioxide test. Ten Negro patients who showed no sickling on the standard sealed tests gave negative results with the carbon dioxide method; the negative findings in this latter group indicate that the procedure of the new test does not of itself cause sickling.

The number of susceptible cells that sickled under the conditions of the carbon dioxide method varied somewhat from case to case. In most instances it was estimated that a minimum of from 20% to 30% sickle cells was present, but in two of our positive cases lesser numbers were found. When present, sickling was always obvious and unequivocal. In all positive cases a conspicuous number of erythrocytes that did not sickle showed distortion and angularity, which was never seen in the negative controls and which evidently represents a