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.

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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

presickle stage. Rouleaux formation was uniformly absent in those cases showing sickling.

It has been established that sickling of susceptible erythrocytes will take place only when the hemoglobin in the cells is in the reduced state (*6, 7, 10*), and the reduction of hemoglobin, however brought about, is the basis underlying all tests for sickling. In the commonly used sealed preparations (*1, 5, 9*), the metabolism of the nucleated blood cells slowly effects the reduction and produces sickling. In the bacteriological test (*11*) the high metabolism of the bacteria brings about more rapid reduction. The use of such agents as BAL, ascorbic acid, etc., depends upon their active reducing properties.

Carbon dioxide, in the technique described, plays a dual role in bringing about hemoglobin reduction. The gas displaces a large part of the air in the flask, thus producing a relatively oxygen-poor environment. In addition, the acid effect of the carbonic acid formed by the reaction of the gas with the blood decreases the affinity of the hemoglobin for oxygen (*2*).

There is little doubt that partial reoxygenation occurs during the transfer of the drop of blood from the flask to the slide. The percentage of cells that are sickled in the final preparation is, therefore, probably less than in the "flask blood" before transfer. It is, of course, possible to prevent this change by fixation of the cells in the flask by the addition of a saline formalin solution before taking a drop of blood for the preparation of the slide. This refinement was not felt to be needed in this test, however, because sickling is easily demonstrated without this step and because a qualitative rather than a quantitative result is all that is necessary.

The rapidity with which sickling can be demonstrated with the carbon dioxide test makes this method of great use in establishing an immediate diagnosis of sickle cell disease or in ruling it out, particularly in emergency situations. A negative reaction conclusively eliminates the possibility of sickle cell disease, whereas a positive reaction indicates that sickle cell disease is to be considered in the differential diagnosis. The simplicity of the test makes the procedure routinely practicable.

The dependability of the test is demonstrated by the absence of false positive and false negative reactions in the series of cases studied.

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To What Extent Is Oxygen Uptake of the Intact Embryo Related to That of Its Homogenate?[1]

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Recently, much attention has been given to analyses of the functions of constituent parts of living tissues, using homogenate and centrifuge techniques. That minute parts of cells can be isolated and concentrated rather easily was demonstrated clearly by Bensley and Hoerr (*1*). Further improvements in techniques and especially the use of high speed centrifugation have made possible detailed quantitative studies on the composition and functions of constituent parts of the living cell (*3–5*). Most of these investigations, however, have dealt largely with vertebrate tissues, and few if any seem to have been concerned with intact organisms and their homogenates. Considerable data are now available on the developmental history of the embryo of the grasshopper *Melanoplus differentialis*. It seemed of interest, therefore, to use such invertebrate material in further studying functions of cell constituents by homogenate and centrifuge techniques (*2*). Many features of this material make it especially desirable for such work. The embryos, free from yolk and in all stages of development, are easily obtained in large numbers (*2*). The egg is of a cleidoic type and hence is quite independent of the external environment for its food supply; it develops quite readily at room temperatures (25° C). Individual embryos, as well as morphologically and physiologically similar ones, are readily obtained, and from them homogenates are easily made. During the course of its development at 25° C, the embryo goes into a mitotically blocked or diapause state in which metabolic and other cellular activities reach a true basal rate (*2*). After removal of this developmental block, mitosis and other cellular activities are again resumed. A study has been made of the oxygen uptake of embryos, both intact and homogenized, and the results show rather striking properties of this material, as well as some differences from vertebrate tissues similarly treated.

Embryos of known age and temperature history were dissected free of yolk, as previously pointed out (*2*). A phosphate buffered Ringer's solution (pH 6.8) was used as suspension medium. Oxygen determinations were carried out at 25° C in Warburg manometers, using respiration flasks of 5-ml capacity. Intact embryos (100), as well as homogenates made from others of the same group, were run simultaneously. A glass type of homogenizer, as described by Potter and Elvehjem (*6*), and powered by an electric motor, was employed.

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