

The remainder of the water was removed by azeotropic distillation with ethylene dichloride under vacuum (2). A dry but gummy residue (I) was obtained which was exceedingly hygroscopic. While still in the drying flask, residue I was extracted twice, for 1 hr each time, with 50-ml portions of 0.2*N* dl-camphorsulfonic acid (CSA) in dry acetone (3, 4).

The extracts (II) containing amino acids and their derivatives possessing free amino groups were decanted from the insoluble components of residue I, and the amino compounds were precipitated by the addition of triethylamine. The supernatant fluid, which retained the triethylamine salt of CSA (5), was decanted and the precipitate was washed several times with fresh dry acetone. Residual acetone was removed in vacuum, leaving a dry stable powder (III).

Tests on fraction III were strongly positive for glucuronic acid, both with naphthoresorcinol (6) and with the more specific carbazole reaction (7).

Fraction III was dissolved in 100 ml of water and passed through an ion-exchange column (IR-4B, Rohm and Haas), which was prepared by the method of Sanger and Tuppy (8). The column was then washed with deionized water until the washings were negative to ninhydrin, thus indicating that the column was freed of unadsorbed compounds.

The column adsorbate was eluted with 0.1*M* sodium tetraborate or citrate until the naphthoresorcinol test indicated that the elution of the glucuronic acid conjugates was complete (9). The total eluate was evaporated to dryness in vacuum and extracted with diethyl ether to remove interfering substances. Residual ether was removed by evacuation and the remaining solids (IV) were extracted with absolute ethanol. Two fractions were thus obtained, one ethanol soluble (V) and the other ethanol insoluble (VI). Examination of these two fractions by ascension chromatography failed to reveal the presence of free amino acids or free glucuronic acid. However, development of the chromatograms with aniline hydrogen phthalate and alkaline potassium permanganate (10) disclosed a reducing substance.

Portions of fractions V and VI were made 1*N* with respect to HCl and were hydrolyzed by autoclaving at a pressure of 10 lb/in.² for 4 hr. The hydrolysates were evaporated to dryness, redissolved in water, and reevaporated. The process was repeated several times to remove residual HCl. Paper chromatography, as well as microbiological assay procedures, established the presence of glutamic acid with only traces of aspartic acid in the ethanol-soluble fraction, whereas by the same techniques aspartic acid was found mainly in the ethanol-insoluble fraction VI.

In preparing dry urine solids (I), there is the possibility that conditions used may have produced the combination of glucuronic acid with glutamic or aspartic acid. However, a portion of lyophilized urine yielded results identical with those obtained on the product from the azeotropic distillation.

The results indicate the presence of a heretofore unreported type of conjugate in urine. Experiments to establish proof of structure, as well as to determine the amounts excreted, are in progress.

References and Notes

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3. E. V. McCollum and A. A. Rider. *Arch. Biochem. and Biophysics* **40**, 20 (1952).
4. E. V. McCollum, A. A. Rider, and H. Sass. *Proc. Soc. Exptl. Biol. Med.* **72**, 709 (1949).
5. Our experience does not confirm the reports (3, 4) that the ammonium salt of CSA, obtained by utilizing dry ammonia gas as the precipitating agent, is soluble in acetone. However, we have found that the presence of urea has some solubilizing effect on the ammonium CSA salt. Also, use of CSA with protein hydrolysates gives rise to a solubilized ammonium CSA salt.
6. B. Tollens and F. Rorive. *Ber. deut. chem. Ges.* **41**, 1783 (1908).
7. Z. Dische. *J. Biol. Chem.* **167**, 189 (1947).
8. F. Sanger and H. Tuppy. *Biochem. J.* **49**, 463 (1951).
9. It was found that the glutamic acid conjugate was present in greatest concentration in the eluate below pH 7 and the aspartic acid conjugate primarily in the eluate above pH 7.
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Communications

The Broken Spectre

In your issue of January 29 [*Science* **119**, 164 (1954)], there is a letter from Donald M. Black regarding "The Broken Spectre of the Desert View Watch Tower, Grand Canyon, Arizona." Mr. Black is evidently under the impression that this phenomenon, commonly called a "glory," is seen very infrequently. In fact, it has become a very usual observation since flying above the clouds became common. All that is required is that the aircraft be between the sun and a cloud and that the observer can see the shadow on the cloud. The aircraft itself is unnecessary except as a carrier of the observer, since the

phenomenon is backward scattering from the water droplets in the cloud and really surrounds the line from the sun through the observer's eye.

In the older literature that deals with atmospheric phenomena, the glory is not explained theoretically, but an attempt at an explanation is given by H. C. van de Hulst under the title, "A theory of the anticoronae" [*J. Opt. Soc. Am.* **37**, 16 (1947)]. No definite experimental verification of this theory has been made, but it seems probable that it is correct.

I have taken some small-scale color photographs from an aircraft over the ocean, but a complete check could be made only from an extensive program of

photography, including observations of the polarization of the radiation. A knowledge of the droplet size in the cloud would also be necessary.

A. G. SHENSTONE

*Palmer Physical Laboratory
Princeton University
Princeton, N. J.*

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An Inexpensive Inoculation Chamber¹

Plant tissue culturists working in sterile or aseptic atmospheres often do not need large areas in which to work. A small, portable chamber will usually suffice to prevent microorganism contamination to their culture containers. For this purpose, a small transfer cabinet was constructed simply and inexpensively. As can be seen from Fig. 1, it consists of plywood backs and sides and lucite top and hinged, slanting panel.

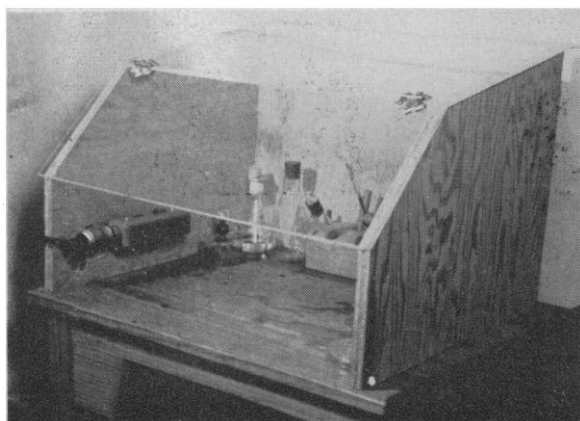


FIG. 1. Portable inoculation chamber.

Provision is made for a small light and necessary materials and working implements; if desired, an ultraviolet lamp may be placed on the back or top near the rear of the chamber. In the case cited, a Vycor No. 830-A, 16-in. ultraviolet lamp is used, attached to the plywood back, just below the lucite top (not shown in Fig. 1).

The cabinet has proved successful in everyday work, both from the standpoint of convenience and aseptic protection. It can be easily moved and adapted to any work area.

More details on the construction and dimensions are given in *Turtox News* (Apr. 1954).

JAMES H. M. HENDERSON
JOHN P. RIER, JR.

*Carver Foundation
Tuskegee Institute, Alabama*

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Geology of the Bedford Shale and Berea Sandstone in the Appalachian Basin

The Berea sandstone of early Mississippian age has been an important source of oil and gas in the Appalachian Basin for many years. This formation and the underlying Bedford shale are exposed at the surface in a long, narrow belt extending from northern Kentucky northward to Berea, Ohio, then eastward into Pennsylvania. From their outcrop, these beds dip east and south under the surface in eastern Ohio, western Pennsylvania, West Virginia, and eastern Kentucky. A study of the Bedford shale and the Berea sandstone at the outcrop and in the records of many thousands of wells drilled to these formations in the search for oil and gas has been completed recently by the U.S. Geological Survey, and the results are now being processed for publication. This study provides an understanding of the paleogeography of the region at the time the rocks were deposited.

In early Bedford time the region was flooded by a shallow arm of an epicontinental sea. This sea arm—the Ohio Bay—covered parts of Ohio, western Pennsylvania, western West Virginia, and eastern Kentucky and was bounded on the west by the Cincinnati arch, on the north by uplands in Ontario, and on the east and southeast by the low-lying Catskill delta of Devonian age.

From the upland in Ontario a vast river, the Ontario, carried sediments that were mainly red into the northern end of the Ohio Bay and deposited them on a delta that built southward. The sediments deposited above water in the main body of this delta retained their red color; those deposited under water were bleached to gray. Along the delta shores waves winnowed the sediments, removed the fine muds, and left the silts in offshore bars. One of these bars, in southern Ohio, is about 80 mi long and 6 mi wide. As the delta built southward, streams carrying mud and silt meandered slowly back and forth across the northern part of the delta and many channels became filled with mud and silt. By late Bedford time, the delta extended southward into northern Kentucky.

At the close of Bedford time, the areas to the north of Ohio Bay in Ontario and to the east in Pennsylvania and West Virginia were unwarped. The Ontario River cut rapidly and deeply into the northern part of the delta and abandoned the southern part by breaking out of the main channel and cutting south-eastward. The sea soon encroached on the southern part of the delta.

In early Berea time, a vast quantity of fine sand was carried into northern Ohio by the Ontario River and deposited in the deep scour channels cut in the northern part of the delta in late Bedford time. These stream channels apparently were filled rapidly by this influx of sand and abandoned, for quarries at Berea and Amherst, Ohio, have quarried stone from these sand-filled channels that lie in Bedford shale, yet few fragments of shale are found within the quarry stone.

On the eastern side of the Ohio Bay, in West Vir-