then spread laterally into the growing pond, the seed floating into the wet area along the pond banks where germination was assured. New growth could not start in the center of the pond, now permanently covered by water. Progressive growths of seedling, sapling, and tree from the periphery toward the center of the pond result in a dome-shaped profile. Where cypress growth started about a permanent pond that was gradually deepening, a cypress doughnut with open water in the center may be formed. Cypresses developing along any natural drainage that ends in a depression as described above will result in the development of a cypress head.

In ponds not connected with artesian aquifers there are three possible causes of the gradual deepening of the water. The basin could be increasing slowly by solution, the basin could be filling with sediment possibly accompanied by subsidence, and the ground-water level could be rising. The uniform development and occurrence of these cypress growths throughout the Peninsula of Florida favors a regional rise of ground-water level and tends to eliminate the other two listed causes, although these may be of local importance. The ground-water surface controlling the water level in these ponds is not artesian, and, as seasonal rises due to heavy rainfall are not permanent, the gradual rise of water level and regional deepening of these ponds and subsequent development of cypress domes, heads, and doughnuts is indicative of a steady rise in sea level throughout the Recent. Because the ground-water surface tends to conform to topography generally, the total rise in ground-water level was proportionally small in relation to the total rise in sea level, and the pond basins filled very slowly.

That the sea level has risen during the Recent has been reported by Davis (*1*), who has described the occurrence of mangrove peats, frequently 10 feet and more in thickness, in the coastal areas of southwestern Florida swamps. Because mangrove growth is limited to within a few inches of the range of the tide, he concluded that these deposits show a rise in sea level, since the present tide range is only three to four feet. Furthermore, in a manuscript describing Florida peat deposits, Davis points out that many lakes contain fibrous peat below a sedimentary clastic peat. As fibrous peat is developed in shallow water marshes and sedimentary peat in deeper water conditions, the water level in the lakes must have risen since the formation of the fibrous peat. This, in turn, could be indicative of a rise in sea level, although other causes must be considered. Such studies as these present a mounting mass of evidence that sea level changes during the Pleistocene and Recent have largely controlled the geomorphology of Florida and adjacent coastal areas. Without considering sea-level changes, it would be difficult, if not impossible, to explain the present physiography, hydrology, soils, and vegetation.

References

1. Davis, John H., Jr. *Pap. Tortugas Lab.*, 1940, **32.**
2. Demaree, Delzie. *Ecology*, 1932, **13**, 258–262
3. Harper, R. M. *Fla. geol. Survey* (18th Ann Rep.), 1927, **27.**
4. Kurz, Herman. *Fla. geol Survey* (23rd-24th Ann. Rep.), 1933, **56.**
5. Vernon, Robert O. *J. Geom.*, 1942, **5**, 302–311.

IN THE LABORATORY

The Concentration and Preservation of Urinary Substances by Lyophilization

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The usual method for concentrating or drying urine is vacuum distillation at elevated temperatures. Its use leaves one in doubt, however, concerning possible changes that may have occurred in the chemical composition of the urinary constituents. Furthermore, the foaming of the urine, resulting from the reduced pressure, interferes with the distillation.

The steroid content of normal urine is relatively low as compared with the amount necessary for its quantitative study. To obviate the difficulties inherent in vacuum distillation, it was thought advisable to investigate the possibility of subjecting large volumes of urine to concentration by lyophilization in order to obtain adequate quantities of steroid substances for analysis.

Urine samples of different concentrations were dried in a lyophil of the type shown in Fig. 1.[1] The lyophil apparatus used in this study was constructed with four equally spaced joints near the base of the jacket of the condenser cone. These joints, placed at a downward angle, were of the short, standard taper 34/45 female type. The male portion of the joints was attached to an 800-cc. Kjeldahl flask. The diffusion path of the water vapor was kept as short as possible.

Not more than 100 cc. of urine in the case of the dilute samples, and proportionally less for the more concentrated ones, are put into each flask of the lyophil. This is frozen in the form of a thin, uniform shell by slowly revolving the flasks in a freezing bath of methyl cellosolve and dry ice. This bath is best contained in an ordinary enameled or agateware saucepan about 7 inches in diameter and 4 inches deep. The freezing is accomplished more uniformly if the bath is cooled substantially before the freezing is started. As soon as the contents of the flask are frozen solid, *i.e.* until the shell of frozen urine cracks or snaps, the flask is transferred to a tray containing more of the methyl cellosolve and dry ice, since all of the flasks must be put on the lyophil in rapid succession. A convenient tray for this purpose is an enameled one approximately 9 x 13 x 2 inches. This will accommodate four of the 800-cc.

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[1] Diagram reproduced from *Science*, 1944, **99**, 285–286, by permission of the authors, D. H. Campbell and David Pressman.

flasks. A vacuum of 0.1–0.2 cm. is maintained in the lyophil by means of a Wegner vacuum pump.

As soon as the samples are frozen, dry ice is added to the methyl cellosolve in the condenser cone of the lyophil. The ice is added cautiously in order to obviate the excessive boiling which is otherwise encountered. No more ice than can be covered by the cellosolve is used at this stage, but the mixture is permitted to become cold before the flasks are put on. The ground-glass joints are well lubricated with a light vacuum grease.[2] The flasks are then placed quickly on the lyophil. Care is taken, however, to observe that each joint becomes transparent in order to be certain of good contact. A sealed-off standard taper is put in place on the joint at the bottom of the apparatus before the freezing is started. During the first 20–30 minutes the lyophil is watched rather carefully until the ice or frost begins to accumulate on the condenser cone. This occurs soon after the peak of foaming in the flasks is reached. Although the condenser cone must be kept as cold as possible during this time, it should not contain too much dry ice because considerable boiling occurs when the condensation begins. After the onset of condensation, the condenser cone should be kept full of dry ice.

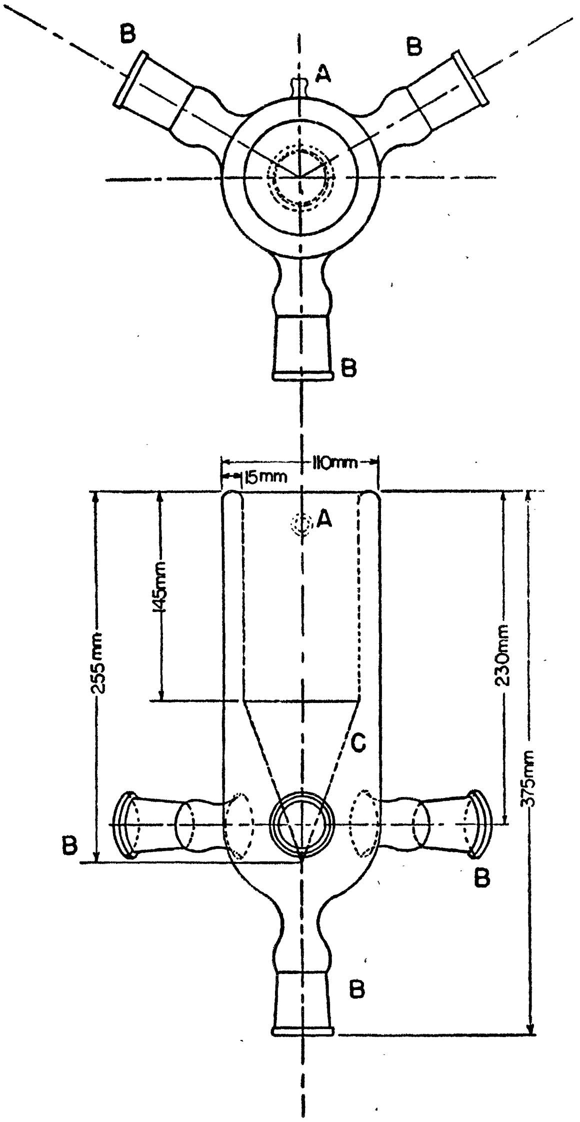

Fig. 1. Diagram of lyophil apparatus showing the side and cross-section views.

Once the ice has formed on the outside of the flasks, the apparatus needs attention only at intervals of approximately one hour. The time of drying depends for the most part on the following factors: (1) size of the sample; (2) concentration of the sample; and (3) temperature and relative humidity of the room. The following results were obtained with urine taken to dryness:

Total volume of samples (cc.)	Specific gravity	Time of drying (hrs.)
100	1.095	9¼
200	1.016	8
400	1.014	10
400	1.006	8
400	1.025	9

This method of concentrating or drying urine of varying concentrations is satisfactory from a number of standpoints: (1) The apparatus is simple and of moderate cost; (2) the process requires little attention once the initial steps are taken; (3) concentration of the urine is done at below-zero temperatures, which are unfavorable to chemical alterations in the constituents of the urine.

The application of the method to the extraction of steroids is now being studied.

Elementary Isotopic Analysis: Determination of Carbon

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We have recently described a new method for the determination of oxygen (*1*) in organic compounds by using heavy oxygen. Using the same method, higher precision is possible in the determination of carbon, since C^{13} is now available (from our two thermal diffusion plants in Marcus Hook) in much higher concentration (10–15 per cent) than the oxygen isotope (1.2 per cent).

In order to demonstrate the increased precision obtainable with our method when using higher isotope concentrations and to extend this method to carbon, we have made the following three carbon determinations on two representative pure compounds, namely, acetic acid (m.p. = 16° C.) and iso-octane (n_{20}^{D} = 1.3916). The $C^{13}O_2$ used had a concentration of 12 atom per cent C^{13}.

A known weight of substance (= a) was burned in a platinum tube with a slight excess of O_2, as described previously (*1*), and simultaneously equilibrated for 30–45 minutes with a known volume of pure isotopic CO_2 (= b). The acetic acid was

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[2] Celvacene-Light Vacuum Grease, Distillation Products, Inc., Rochester, New York.