B complex was fed separately twice daily to each rat except those maintained on the stock diet, which provided an ample natural supply in the yeast. Each vitamin allotment was fed in the form of a pellet containing thiamine, 20 μg.; riboflavin, 30 μg.; and pyridoxine, 30 μg.; calcium pantothenate, 0.1 mg.; nicotinic acid, 0.5 mg.; p-aminobenzoic acid, 1.0 mg.; inositol, 1.0 mg., choline, 10 mg., starch, 50 mg., and corn syrup, 25 mg. Crisco supplied vitamin E.

At 26 days of age, 19 male and 19 female rats of the Sprague-Dawley strain were placed on the diets outlined, the same number of males as of females on each. After 20 days (2 days longer than the longest period of Keller), all of the animals were transferred to the stock diet, and the separate feeding of the synthetic vitamin B complex mixture was discontinued. At 100 days of age, male and female rats which had been given the same diet during the initial 20-day period were paired off for mating. To make sure that the supply of vitamin E was adequate for implantation and gestation, 15 grams of wheat-germ oil were added to each kilogram of stock diet. Table 2 summarizes the observations made on growth in the 20-day deprivation period and indicates the number of young in the litters resulting from each mating.

TABLE 1

COMPOSITION OF TRYPTOPHANE-DEFICIENT, CONTROL, AND STOCK DIETS*

	G (grams)	Gt (grams)	Gvt (grams)	Hy (grams)	Hyt (grams)	Pr-free (grams)	Stock (grams)
Bacto-gelatin	30.0	29.8	28.4				
Tryptophane-deficient casein hydrolysate				17.7	17.5		
Casein							17.7
l-Cystine				0.3	0.3		0.3
l-Tryptophane		0.2	0.2		0.2		
dl-Valine			1.4				
Starch	25.0	25.0	25.0	37.0	37.0	55.0	29.0
Yeast							8.0

* Diets other than the stock diet are designated by symbols which suggest their nitrogen composition: G, gelatin; Hy, casein hydrolysate; t, supplementation with tryptophane and v, with valine; Pr-free, protein free. Per 100 grams, each diet also contained glucose, 15 grams; Crisco, 19; cod-liver oil, 5; salt mixture (*2*), 4; and agar, 2. The B-complex vitamins were provided in pellets as described in the text.

TABLE 2

AVERAGE GROWTH DURING THE 20-DAY PERIOD ON THE VARIOUS DIETS TOGETHER WITH SUBSEQUENT REPRODUCTION HISTORY

Diet fed during the 20-day period	G	Gt	Gvt	Hy	Hyt	Pr-free	Stock
Pairs of rats tested	4	3	2	4	2	2	2
Avg. initial wt. per rat (grams)	43.1	42.0	41.5	43.7	45.0	47.0	43.0
Avg. change in wt. per rat (grams)	−13.6*	0.0	+1.0	−11.7	+44.0	−12.0	+79.0
Size of litters†	11, 13 10, 11 5, 7	10 9 8	6 6	10, 7, 8 8, 11 7, 14 6, 10	14 10	9 8	11 8

* One of the females on the unsupplemented bacto-gelatin diet died on the 20th day.

† One of the females that had been on the tryptophane-deficient diet, Hy, was mated three times and bore three litters; all others that had been on diets G and Hy were mated twice and bore two litters.

During the 20 days on the experimental diets, the animals on the tryptophane-deficient hydrolysate (diet Hy) lost about one-fourth of their initial weights. The fact that this loss was essentially the same as that in the rats on the protein-free diet (Pr-free) indicates that the casein hydrolysate was markedly deficient; stimulation of growth by the incorporation of tryptophane (diet Hyt) affords evidence that the hydrolysate lacked chiefly this amino acid (*1*). The weight loss on the unsupplemented gelatin diet (G) was slightly, but possibly not significantly, greater than on the other tryptophane-deficient diets. Supplementation of gelatin with tryptophane (diet Gt) or with both tryptophane and valine (diet Gvt) prevented the loss in weight but did not promote significant growth in the 20 days. This supports our earlier implication that it is quite unfair to assume that unfavorable responses on gelatin diets may be attributed, without question, to their deficiency in tryptophane.

The reproductive performance of the pairs of rats which had been fed the tryptophane-deficient diets (G, Hy, and Pr-free) compared very favorably in every way with that of the control pairs fed supplements of tryptophane (Gt, Gvt, and Hyt), as well as with that of the control pairs fed the stock diet throughout.

We are not prepared to account for the observations reported by Keller, but the tests recorded in this communication convince us that a period of *uncomplicated* tryptophane deficiency in young rats, lasting as long as 20 days, *does not induce subsequent sterility.*

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Cypress Domes

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The extensive flatlands of the peninsular area are a monotonous part of the Florida landscape, characterless except for vegetation growths. One of the most unique of these are large stands of cypress that appear, at a distance, to be buttes standing out against the horizon (Fig. 1). These stands of cypress occur in dome-like masses, with the taller trees growing in the center and those toward the periphery being successively shorter (Fig. 2). Some of these groves are of immense proportions, being up to a mile in diameter. The map appearance of the dome may be round, elliptical, or, in rare cases, there may be open water in the center, the dome appearing as a doughnut. Both species of cypress are present in these stands, *Taxodium distichum* (L.) Richard, for example, growing along the St. Johns River Valley, and *T. ascendens* Brongn, in the Big Cypress of the southern Peninsula. The uniform occurrence of these domes throughout the Peninsula of Florida suggests a close relationship to the geomorphology of Florida and is therefore of interest to others than the botanist.

These cypress domes were discussed by Harper (*3*), who thought that the trees nearest the periphery were dwarfed by unfavorable soil conditions and believed that the small trees were as old as the large. Kurz (*4*) proved, by counting the tree rings, that the trees were progressively younger toward the

FIG. 1. Cypress domes on prairie near Deer Park, west of Holopau, Florida. (Photograph taken by John H. Davis, Jr., from highway U. S. 192.)

outside of the dome. He explained the dome shape by an increasing premature mortality rate in the cypress from the center of the dome to the outside. He was unable to explain just why cypress "should die back partly or altogether prematurely in the shallower water where growing conditions should be best." Likewise, a high mortality rate progressively nearer the periphery does not explain why older trees are not scattered throughout the dome, as would be expected.

Plant ecologists have proved that cypress seed will not germinate when covered with water, and Demaree (*2*) showed that, if it is to sprout, the seed must lie on land that is not submerged, and the seedling, to survive, must grow enough to keep above seasonal water rises. Demaree cites the case of Reelfoot Lake in Tennessee, the basin of which was suddenly created by the New Madrid earthquake of 1811–12, as proof that even old and established cypress trees will be drowned if they are rapidly submerged in water. Experiments conducted

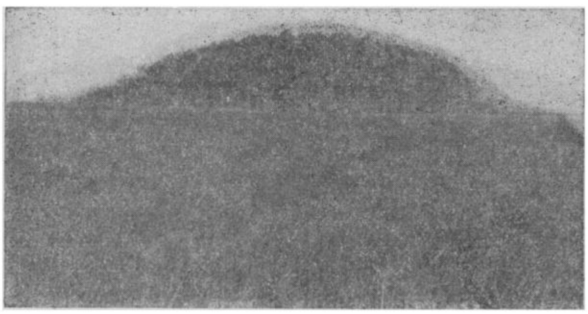

FIG. 2. Bald cypress (*T. distichum*) dome west of Melbourne, Florida. (Photograph taken by Herman Kurz.)

by him in 1928–29 on the effect of drowning on cypress seed and seedling support his Reelfoot Lake observations, that a growing cypress tree or seedling will die if it is suddenly submerged in water. He did not attempt to study the effect of gradual drowning on cypress, but observations made by the writer in 1943 indicate that, where submergence is slow and gradual, the cypress tree is able to adjust, in moderation, to deeper water, even where this water becomes permanent. In western Florida, where the more rapid alluviation in main valleys has caused the ponding of tributary streams and the formation of lakes in the lower parts of the tributary valleys, the trees growing on the flood plains and marginal swamps were slowly drowned (*5*). These lakes mark an advancing regional filling of previously eroded valleys, and the ponding dates back to the start of the Recent and is indicative of a rising sea level that has initiated valley filling. Thus, ponding and subsequent drowning of the trees growing on the flood plains of the tributary streams was very gradual, and the cypress occurring in these lakes record the reaction of cypress to slow drowning. Dead Lake, the drowned lower portion of the Chipola River, is studded with cypress trees and stumps and is an excellent illustration. In a line starting at the Chipola River channel, near the center of the lake, and running to the lake banks, the general succession of tree vegetation is (1) partially submerged and rotted stumps, (2) partially rotted stumps extending above water, (3) decayed trees still retaining dead branches and an occasional live branch, (4) vigorous mature growth, (5) saplings, and (6) seedlings (Fig. 3). The stumps near the channel have buttresses that extend above the present lake level, and some of these now stand in 25 feet of water. The cypresses have thus been able to adjust to, and build buttresses in, water depths of at least 25 feet before being killed, and the succession of development of trees indicates that new trees have worked continuously toward the banks of a slowly spreading and deepening lake.

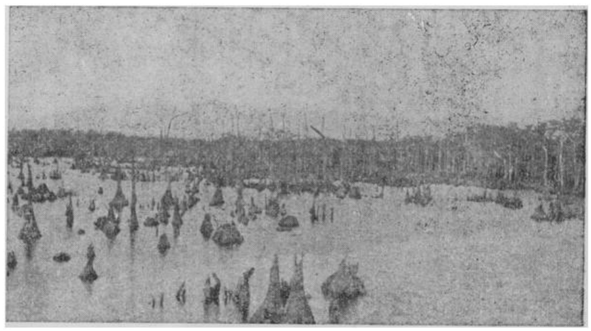

FIG. 3. Succession of cypress growth in the south end of Dead Lake, along the lower part of the Chipola River, Gulf County, Florida. (Photograph taken from the channel.)

Stands of cypress where the tallest trees grow in the center and shorter trees grow outside are called domes because of the cross-section profile when viewed from the side. Where an elongated growth of cypress with linear dimensions ends in a dome, the dome is referred to as a cypress head, and if open water is present in the center the growth is called a cypress doughnut.

The observations made in Dead Lake and on the cypress domes of the Peninsula of Florida have led the writer to believe that these domes were started in seasonal ponds or swamps, where the seed sprouted when not submerged, the seedling then growing sufficiently high to stay above seasonal rises in water level. The subsequent development of the dome is most easily explained by a gradually deepening pond, the basin filling so slowly that the biologic changes, necessary for the cypress to adjust to the changing habitat, could keep pace. In such a seasonal pond cypress trees developed and grew and 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.

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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.

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