reflects a relationship with early North American technology.

Samples of the obsidian are being submitted to the Smithsonian Institution obsidian-dating project, but definitive samples must await the major excavation planned for the summer of 1960.

El Inga is considered to be the camp and workshop site of some of South America's earliest men. The inhabitants were probably part of the first wave of migrants from North America, and would thus predate the Fell's Cave people by a thousand years or more. In this interpretation it is quite likely that early North American styles and traits would be most prominent in northwestern South America.

WILLIAM J. MAYER-OAKES
ROBERT E. BELL
University of Oklahoma Museum, Norman

Reference

1. J. B. Bird, *Geographical Rev.* **28**, 250 (1938).

2 March 1960

Response of Cupressus funebris Tissue Cultures to Gibberellins

Abstract. Gibberellins are a specific growth requirement of tissue cultures derived from the staminate cones of *Cupressus funebris*. The tissue originally required coconut milk for growth. However, a mixture of acid-hydrolyzed casein hydrolyzate and gibberellins replaces the coconut milk entirely and permits even better growth. It is tentatively suggested that coconut milk contains gibberellins or related substances and that these substances are involved in the nitrogen metabolism of the tissue.

Accounts of the effects of gibberellins on intact plants have been accumulating for several years. Stowe and Yamaki (*1*) have recently written two reviews concerned with this literature. Generally, the response of intact plants to gibberellins have been quite striking. These responses are most commonly manifested by spectacular increases in stem length, especially of certain genetic dwarfs, and in flower production by long-day plants with the substitution of gibberellins for the inductive photoperiod.

Attempts to elicit responses of tissue cultures to gibberellins have been rather disappointing. Netien (*2*) tested the effects of gibberellins on tissue cultures of *Helianthus tuberosus, Rubus sp., Daucus carrota*, and crown-gall tissue of *Scorsonera sp.* and observed that there was either no effect or a slight inhibition of growth. Various types of auxin used in conjunction with gibberellins did not change the response appreciably. Henderson (*3*) used tissues of *Helianthus annuus* as the test material and found no appreciable response to gibberellins. Nickell and Tulecke (*4*), in an extensive series of tests utilizing 49 strains of tissue cultures representing 25 species, found that inhibition of growth was the most common result of the incorporation of gibberellins into the nutrient medium. Two tissues died, while several showed a slight increase in weight over the controls. Schroeder and Spector (*5*) reported a synergistic action of gibberellins and indoleacetic acid in the induction of callus growth in explants of mature citron fruit tissue. Gibberellins alone were about one-third as effective as a mixture of gibberellins and indoleacetic acid. The greatest increase in fresh weight was about 100 percent at the end of the experimental period. It can be seen from this short review that plant tissue cultures in general have not been reported to respond very markedly to gibberellins.

The present report is concerned with a spectacular growth response to gibberellins by tissue cultures derived from the staminate cones of *Cupressus funebris*. These tissues were originally isolated by C. D. LaRue in 1954. After his untimely death in 1955, one of us (J. S.) acquired the tissues from his laboratory. The tissues were maintained on White's medium containing 20 percent (vol./vol.) autoclaved coconut milk obtained from mature nuts.

During investigations of certain aspects of the carbohydrate metabolism of the tissue, it became imperative to grow the tissue on a synthetic medium, or at least one which did not include unknown soluble carbohydrates. Consequently, attempts were made to replace the coconut milk with acid-hydrolyzed casein hydrolyzate, indoleacetic acid, adenine, kinetin, and 1,3-diphenylurea. These were used alone and in various combinations. None of the media so prepared permitted much growth of the tissue. However, when a medium containing acid-hydrolyzed casein hydrolyzate (2 gm/liter) and gibberellins (1 mg/liter) was tested, the tissue grew very well. Table 1 summarizes the data obtained with varying concentrations of gibberellins (*6*).

Concentrations of gibberellins between 0.5 and 2.0 mg/liter in conjunction with acid-hydrolyzed casein hydrolyzate permit better growth than coconut milk. Increased concentrations of gibberellins are somewhat inhibitory. Indoleacetic acid was tested for a possible synergistic action with gibberellins. A single concentration of indoleacetic acid of 0.5 mg/liter was tested with media containing different concentrations of gibberellins. In each case, the growth of the tissue was inhibited. The inhibition caused by indoleacetic acid ranged from 40 percent when used in conjunction with 0.1 mg of gibberellins per liter to 58 percent when added to media containing these substances at 2 mg/liter.

Table 1. The effect of concentrations of gibberellins on the growth of tissue cultures of *Cupressus funebris*. CH, acid-hydrolyzed casein hydrolyzate; GB, gibberellins. Concentration of CH in all cases was 2 gm/liter. The basal medium consisted of White's solution, 2 percent sucrose, and 0.9 percent agar.

Addendum to basal medium	Increase in fresh weight after 35 days (%)
None	84
Coconut milk, 20 percent (vol./vol.)	912
CH	228
GB, 1.0 mg/liter	260
CH + GB, 0.10 mg/liter	620
CH + GB, 0.50 mg/liter	943
CH + GB, 1.0 mg/liter	1003
CH + GB, 2.0 mg/liter	1004
CH + GB, 10.0 mg/liter	720
CH + GB, 20.0 mg/liter	538

It is tempting to speculate that gibberellins are somehow concerned with the nitrogen metabolism of the tissue. Without any organic nitrogen added to the medium, gibberellins at 1 mg/liter permit three times the amount of growth obtained on basal medium alone. Acid-hydrolyzed casein hydrolyzate added to the basal medium permits almost as good growth as gibberellins alone. Thus, while the tissues appear to be unable to utilize the inorganic nitrogen in the basal medium, under the influence of gibberellins some utilization is possible. The amount of growth under these circumstances may be limited by the low concentration of nitrate in White's medium (approximately 3.2 mmoles of nitrate ion). We have found that 2 gm per liter is the optimal concentration of the hydrolyzate for these tissues. It would seem, then, that gibberellins enhance the utilization by the tissue of both organic and inorganic nitrogen sources.

One of the implications of this work is that gibberellins or related substances are responsible for some of the growth-promoting activity of coconut milk for these tissue cultures, at least. Radley and Dear (*7*) have shown that extracts of the milk, solid endosperm, and embryo of the coconut accelerate the growth of dwarf peas. They therefore suggest that coconuts contain gibberellin-like substances. In addition, when one considers the report of MacMillan and Suter (*8*), which announced the isolation of gibberellin A from young seeds of the runner bean, and the specific response to that gibberellin by

certain genetic dwarfs of maize and peas (*9*), the probability that higher plants contain and also require gibberellins for growth and development is strengthened.

This work also supports the thesis (*5*) that gibberellins may be involved in cell division. The great increase in the amount of tissue must involve considerable cell division. Without gibberellins and a source of organic nitrogen, growth of the tissue is severely limited.

Cupressus cultures are the only tissue culture which have thus far been demonstrated to require gibberellins in order to attain what is considered to be maximum growth in vitro. It is doubtful that this requirement is associated with the fact that the tissue is of gymnosperm origin, since Nickell and Tulecke (*4*) used pollen-derived tissues of *Ginkgo* and *Taxus* in their extensive study and found no very great response to gibberellins. Ball (*10*), Reinert and White (*11*), and Barnes and Naylor (*12*) have grown tissue cultures derived from various gymnosperms on synthetic media which do not contain gibberellins. In addition, we have tested the effects of these substances on tissue cultures derived from the staminate cones of *Libocedrus decurrens* and have not observed any appreciable effects which may be attributed to them.

Because of their specific response to gibberellins, tissue cultures of *Cupressus* may be an excellent tool with which to study some of the mechanisms of action of these compounds. Tissue cultures permit very close control of environmental, nutritional, and genetic variables, an advantage not afforded by intact plants.

JACOB STRAUS
RODNEY R. EPP
Department of Biology, University of Oregon, Eugene

References and Notes.

1. B. B. Stowe and T. Yamaki, *Ann. Rev. Plant Physiol.* **7**, 181 (1957); *Science* **129**, 807 (1959).
2. G. Netien, *Compt. rend.* **244**, 2732 (1957); *ibid.* **247**, 1645 (1958).
3. J. M. H. Henderson, *Nature* **182**, 880 (1958).
4. L. G. Nickell and W. Tulecke, *Botan. Gaz.* **120**, 245 (1959).
5. C. A. Schroeder and C. Spector, *Science* **126**, 701 (1957).
6. The gibberellins used in this investigation were obtained from the California Corporation for Biochemical Research and are designated as grade B. The mixture consists of unspecified proportions of gibberellins A and X (gibberellic acid).
7. M. Radley and E. Dear, *Nature* **182**, 1098 (1958).
8. J. MacMillan and J. P. Suter, *Naturwissenschaften* **45**, 46 (1958).
9. B. O. Phinney, *Proc. Natl. Acad. Sci. U.S.* **42**, 185 (1956); P. W. Brian and H. G. Hemming, *Physiol. Plantarum* **8**, 669 (1955).
10. E. Ball, *Growth* **14**, 295 (1950).
11. J. Reinert and P. R. White, *Physiol. Plantarum* **9**, 177 (1956).
12. R. L. Barnes and A. W. Naylor, *Botan. Gaz.* **120**, 63 (1958).

18 February 1960

Estrogen-like Activity in Vegetable Oils and Mill By-products

Abstract. By the immature female mouse bioassay technique, an increased uterine weight was observed when certain vegetable oils were fed or injected. By-products from the milling of cereals were also capable of eliciting a uterine response.

In the course of evaluation of the estrogenic potency of the coumarin derivative coumestrol (*1*), administered subcutaneously in vegetable oil solution, it was observed that the vegetable oils employed as carriers were themselves capable of eliciting a measurable response in the test animals. Estrogenic activity has been previously reported in wheat germ oil (*2*) and coffee oil (*3*). We have now found that a number of the commonly used vegetable oils are capable of producing estrogen-like responses in mice.

The bioassay employed in our laboratory for measuring estrogenic activity when estrogen is administered orally has been recently described (*4*). In our subcutaneous injection studies, the estrogen, dissolved in a suitable vegetable oil carrier, was administered for four successive days at the rate of 0.1 ml of oil solution per day; the mouse was killed on the 5th day, and the uterine weight was determined as described previously (*4*).

The vegetable oils employed in these studies included samples of commercially available salad oils as well as some unrefined or semirefined samples. Samples of mineral and cod-liver oils were also included for comparison. For oral administration, the oils or cereal fractions were uniformly mixed into the basal feed mixture at levels equivalent to 10 or 20 percent of the diet. The animals were fed in groups of five, and the results presented (Tables 1–3) represent the averages for five animals. Successive repetitions of the test gave very similar results.

The results of oral administration of the oils are summarized in Table 1. Control mice fed no oil beyond that normally present in the stock diet had an average uterine weight of about 9.5 mg at the end of the test period. Some of the oils tested at the 10-percent level of the diet produced uterine weights more than double this value, while others produced lesser effects. Cottonseed, mineral, and castor oil had no effect. The crude coffee oil and isano oils proved toxic, and the mice died before the test was completed. When the oils were administered at the 10-percent level of the diet, each mouse consumed a total of 1 gm of the oil during the period of the assay. Corn oil was slightly more effective when injected (Table 2) than when administered orally. Olive oil was also effective when it was injected subcutaneously.

As has been pointed out by Biggers (*5*), uterine response to oral or subcutaneous administration of a substance is not necessarily indicative that the substance is an estrogen. A true estrogen is also capable of producing vaginal cornification. Levin *et al.* (*2*) were able to demonstrate that a concentrate obtained from wheat germ oil by the Girard-Sandulesco reaction (*6*) produced vaginal cornification in mature, ovariectomized rats.

Since oils from cereal sources showed activity, it was decided to check unextracted cereal products. When wheat

Table 1. Mouse uterine weight response to various oils administered orally. The oils were fed at the 10-percent level unless otherwise indicated.

Kind of oil	Level fed (%)	Mean uterine wt. (mg)
None (control diet)		9.5
Mineral oil	10	8.6
Castor oil, refined	10	9.4
Cottonseed oil, refined	10	10.1
Safflower oil, refined	10	13.6
Wheat germ oil, refined (sample 1)	10	13.6
Cod-liver oil, refined	10	13.9
Corn oil, refined	10	14.2
Corn oil, refined	20	15.8
Linseed oil	10	14.6
Wheat germ oil (sample 2)	10	15.0
Peanut oil, refined	10	15.9
Olive oil, refined	10	16.7
Soybean oil, refined (sample 1)	10	16.8
Soybean oil, refined (sample 2)	10	17.7
Coconut oil, crude	10	19.0
Rice bran oil, refined	10	22.5
Rice bran oil, crude	10	23.1

Table 2. Mouse uterine weight response to vegetable oils injected subcutaneously. Four 0.1-ml injections were given in each case, unless otherwise indicated.

Material	Total vol. (ml)	Mean uterine wt. (mg)
None		9.9
0.9% Saline	0.4	10.3
Corn oil (sample 1)	0.4	13.2
Corn oil (sample 1)*	0.8	18.2
Corn oil (sample 2)	0.4	16.8
Corn oil (sample 3)	0.4	15.1
Olive oil	0.4	12.5

* Eight 1-ml injections.

Table 3. Mouse uterine weight response to cereal fractions fed at the 10-percent level.

Fraction	Mean uterine wt. (mg)
No supplement	9.5
Wheat bran	14.3
Wheat germ	25.6
Rice bran	18.3
Rice polish	21.3