

the next. This experience agrees with that reported by Johnson and his co-workers for calves on purified diets. As Mead and Goss⁶ limited food consumption to avoid bloat, they had no critical measure of the appetites of their animals. In ewes this difference in food consumption was not manifest.

(4) The animals had depraved appetites, manifested by wood chewing. This condition was observed in both ruminants and was not evident when finely ground alfalfa was replaced by whole alfalfa. Although others^{2, 10} have observed this phenomenon when ruminants were limited to concentrates, it has not been attributed previously to the physical nature of the diet. Since the animals were fed all they would consume, hunger in the usual sense of the term was not involved.

The lack of coarse material in the diet appeared to produce more pronounced symptoms in cattle than in sheep. Possibly, then, the former require coarser feeds. As is well known, cattle consume coarse, stemmy roughages more readily.

These results show that a physical deficiency, a lack of coarse irritating material in the rumen, in the ration of ruminants results in the following syndrome: failure of, or diminished, rumination; difficulty in eructation, often causing tympany or bloat, especially in cattle; reduction in food consumption in cattle; and depraved appetite, as manifested by wood chewing.

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THE CULTURE OF YOUNG CONIFER EMBRYOS IN VITRO

CULTURE of embryos *in vitro* has been made by many workers in the past, the more important works on the subject are those of Dietrich,¹ Li,² LaRue,³ Tukey,⁴ Bonner,⁵ Solacolu, *et al.*⁶ and van Overbeek, *et al.*⁷ The materials used by these workers are mature or immature plant embryos which had already attained considerable size. As far as we are aware, no successful attempt has been made to culture very young embryos of one to several cells in size. The present account is a preliminary report of our work, which is

¹⁰ L. L. Madsen, C. M. McKay and L. A. Maynard, *Cornell Univ. Agr. Exp. Sta. Mem.* 178, 1935.

¹ K. Dietrich, *Flora N. F.*, 17: 379, 1924.

² T. T. Li, *Sci. Rept. Nat. Tsing Hua Univ.*, Ser. B. 2: 29, 1934.

³ C. D. LaRue, *Am. Jour. Bot.*, 22: 914, 1935; *Bull. Torrey Bot. Club*, 63: 365, 1936, and 65: 11, 1938.

⁴ H. B. Tukey, *Jour. Hered.*, 24: 1, 1933; *Am. Soc. Hort. Sci.*, 32: 313, 1934; *Bot. Gaz.*, 99: 630, 1938.

⁵ J. Bonner, *Plant Physiol.*, 13: 865, 1938; *Proc. Nat. Acad. Sci.*, 24: 70, 1938.

⁶ T. Solacolu, *et al.*, *Compt. Rend. Soc. Biol.*, 129: 403, 1938.

⁷ J. van Overbeek, *et al.*, *SCIENCE*, 94: 2441, 350, 1941.

being continued, on the culture of isolated conifer embryos when the latter are only at the one- or several-cell stage.

Embryos of pine (*Pinus yunnanensis*) and Keteleeria (*K. Davidiana*) were dissected out from the ovules and transferred to culture media in Petri dishes under aseptic conditions. The medium used is a modified Pfeffer's solution⁸ containing 2 per cent. sucrose and 0.6 per cent. agar. To this "standard" medium (SM) was added various growth substances (heteroauxin, 10 mg per liter; thiamin, 0.1 mg per liter; ascorbic acid, 10 mg. per liter; nicotinic acid, 1 mg per liter or vitamin B₆, 0.1 mg per liter).

Young embryos⁹ of pine were inoculated on agar media containing SM and SM and added growth substances on June 11, 1942. After two weeks, the one- to several-celled embryos grown on SM had less than ten cells, while that grown on SM plus indol-acetic acid developed into an embryo of several hundred cells in size, and that grown on SM plus thiamin attained a size of about one hundred cells. Later observations, made on and after July 29, revealed but little growth in any of the cultures.

Keteleeria embryos were cultured in the same manner on July 7. In one of the cultures containing SM plus indol-acetic acid and thiamin a 2-celled embryo of 60 × 40 μ became a 12-celled embryo of 156 × 117 μ ten days later, and this developed into a multicelled embryo of 608 × 156 μ on July 25. Although young embryos of Keteleeria can develop somewhat on all the media used, none of these can approach SM with indol-acetic acid and thiamin as a favorable medium for the growth and development of Keteleeria embryos.

Further work on this problem is necessarily postponed due to seasonal limitations, but these preliminary experiments indicate that it is probable that conifer embryos of one to several cells in size can grow and develop normally *in vitro* in the presence of heteroauxin and thiamin, at least for a certain length of time. It is possible that they may even be grown to maturity *in vitro* with medium containing other growth factors in addition to heteroauxin and thiamin.

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⁸ One liter of this solution contains: Ca(NO₃)₂, 328 mg; KNO₃, 80 mg; MgSO₄ · 7 H₂O, 98 mg; KH₂SO₄, 13 mg; KCl, 74 mg; FeCl₃, 1.6 mg.

⁹ Young embryos of pine undergo cleavage soon after the elongation of their suspensors, so that each embryo is split into four single-celled embryos.

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