by treating bovine plasma with 0.5 N sodium hydroxide at 37° C. for periods longer than 8 hours were toxic when injected into guinea pigs, although such products were non-antigenic by the criteria employed.

We have tested also preparations isolated after treatment of bovine serum with 1 N sodium hydroxide at room temperature for periods of 1 hour, 2 hours, 4 hours and 8 hours. None of these preparations appeared to be toxic when administered intravenously to guinea pigs in doses as large as 300 mg per kg. The materials isolated after 1 hour and 2 hours (but not those isolated after 4 hours and 8 hours) gave weak precipitin tests with an antiserum prepared by injecting untreated bovine serum into rabbits. These same materials gave negative tests with other potent antisera prepared in the same way. However, we have been successful in preparing antisera to each of these 4 materials by intermittent intravenous injection into rabbits for periods of from 21 to 99 days. It is interesting that untreated bovine serum and all 4 of the alkali-treated materials gave positive precipitin tests with each of the antisera prepared with the alkali-treated proteins. Guinea pigs passively sensitized with antiserum prepared with protein isolated after treatment of serum for 2 hours with alkali were shocked with each of the other alkali-treated preparations (except the preparation treated for 1 hour, which was not tested) and with untreated bovine serum.

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A PHYSICAL DEFICIENCY IN THE RATION OF RUMINANTS

THAT ruminants do not thrive on a ration limited to concentrates is well known. Several investigators have attempted to determine the deficiencies associated with such a diet.[1,2,3,4,5,6] Mead and his coworkers[4,5,6] found that calves could be carried through growth, pregnancy and lactation if cod-liver oil and calcium carbonate were added to a common concentrate mix. These animals could not, however, be considered normal, for it was necessary to limit their food consumption to avoid bloat. Despite precautions, four of the animals died of this cause. Johnson and his associates[7] fed calves a purified diet, adding Cellophane to make the ration more bulky. They state: "The growth rates of the 15 calves studied were below normal in most cases in comparison with Ragsdale's Standards. Poor food consumption associated with periodic digestive upsets seemed to be largely responsible for the slow growth."

As we have duplicated several of the symptoms observed on a diet of concentrates simply by fine grinding of roughage, it appears that a concentrate diet has physical as well as chemical or nutritive deficiencies. Several workers have considered that the mere lack of bulk might be a limiting factor in a concentrate diet for ruminants. The addition of soft bulky material such as paper pulp[6] or Cellophane[7] had, however, little beneficial effect. Rather, the limiting physical factor in a concentrate diet appears to be the absence of the coarse sharp material necessary to stimulate nerve fibers terminating in the ruminal mucosa. In experiments with cattle and sheep, finely ground alfalfa hay was compared with whole alfalfa, each being given in conjunction with concentrates. The concentrates and hay were fed in approximately equal proportions. The concentrate mixture consisted of rolled barley 60 per cent.; wheat bran 25 per cent.; soybean meal 12.5 per cent.; NaCl 2.5 per cent. The animals received all they would consume over a feeding period of approximately 8 hours. Since the chemical constituents of the two rations were the same, any discrepancies in response should depend upon the physical condition of the feed—whether ground or whole.

The following disorders were noted only when finely ground alfalfa was fed:

(1) Rumination occurred very irregularly or not at all. This is in line with the finding of Schalk and Amadon[8] that coarse material in the rumen stimulates rumination. Fine grinding so effectively breaks down the alfalfa stems that their irritating effect is largely lost.

(2) Bloat occurred frequently in cattle, but only once in a sheep. Twenty-one cases of bloat were encountered in 4 cows receiving the ground alfalfa hay over a 15-day period. This finding supports the postulate[9] that bloat results from a lack of sufficient coarse irritating roughage to induce the eructation reflex.

(3) Food consumption in cows was reduced as compared with consumption of whole alfalfa. This reduction amounted to 6.9 pounds daily per animal. The reduced consumption by cows receiving finely ground hay was largely due to the fact that, after consuming a large amount on one day, they were off-feed

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the next. This experience agrees with that reported by Johnson and his co-workers for calves on purified diets. As Mead and Goss[6] 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 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 asceptic conditions. The medium used is a modified Pfeffer's solution[8] 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 B_6, 0.1 mg per liter).

Young embryos[9] 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 $60 \times 40\ \mu$ became a 12-celled embryo of $156 \times 117\ \mu$ ten days later, and this developed into a multicelled embryo of $608 \times 156\ \mu$ 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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[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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