

tion of "Coenzyme R" obtained from *Azotobacter* by the method of Hoover and Allison³ was tested, and it was found, in agreement with these workers, that alone it was completely inactive with yeast. When 0.1 gamma per ml of β -alanine was added, however, the preparation stimulated yeast growth in a manner identical with that of the biotin concentrate. For maximum growth response, it required 5 gammas per ml of the former, but only 0.3 gamma of the latter. With a given level of either "Coenzyme R" or biotin concentrate, a maximum in the curve of yeast stimulation was obtained in the region of 0.05 to 0.5 gamma per ml of β -alanine. With *R. trifolii* near maximum stimulation was obtained with either "Coenzyme R" or biotin alone; addition of 0.05 gamma per ml or over of β -alanine gave a small but consistent supplementary effect. It is not surprising that β -alanine is much less effective with *R. trifolii* than *S. cerevisiae* since the former is apparently capable of decarboxylating aspartic acid to form β -alanine.⁴ The growth of *R. trifolii* was not decreased when the amount of β -alanine was raised to 1.0 gamma per ml. These results have been confirmed with several species of *Saccharomyces* and *Rhizobium*.

Consideration of these data leads to the following alternative conclusions:

- (1) "Coenzyme R" and biotin are identical.
- (2) "Coenzyme R" and biotin are distinct, but with such similar chemical and physical properties that we have not been able to separate them by any of the procedures indicated.
- (3) "Coenzyme R" and biotin are distinct, but each acts as a growth stimulant for either or both yeast and rhizobia.

Final decision with regard to the relationship must necessarily rest until both factors are available in pure form. However, until a more purified "Coenzyme R" is prepared which definitely shows no activity for yeast even in the presence of β -alanine, conclusion 1 appears to be the simplest assumption. If the two factors are distinct, the present evidence favors conclusion 3, *viz.*, that biotin also is a stimulative factor for *R. trifolii*.

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SAND CULTURE¹ OF COTTON PLANTS

THE successful culture of many vegetables and flowers in water, sand, cinders, etc., suggested that

this technic might be useful in research on the physiology of the cotton plant. Publications on the subject have been scattered in scientific and lay journals; in those available to us, no references to the culture of cotton plants were found.

Preliminary attempts to grow cotton seedlings by immersion of the roots in nutrient solutions were not successful. However, when the young plants were placed in sand,² they grew to maturity. The seeds were germinated between moistened filter papers and when the roots were from 1½ to 2 inches long, each was transplanted to a washed clay flower pot (4-inch diameter) containing wet sand. The sand was kept wet by irrigation twice daily with distilled water. Every third day, the sand was flooded with the culture solution. The solution used was that recommended by the New Jersey Agricultural Experiment Station as described by Ellis and Swaney,³ and contained the following ingredients⁴ dissolved in distilled water:

	Parts per million
Ca(NO ₃) ₂	740
KH ₂ PO ₄	310
MgSO ₄	280
(NH ₄) ₂ SO ₄	95
FeCl ₃	8.5
H ₃ BO ₃	0.85
ZnSO ₄	0.48
MnSO ₄ ..	0.46
CuSO ₄	0.07

The pots were kept on the window sills of a chemical laboratory with southern exposure. On clear days, they received approximately two hours of sunshine. Despite these abnormal environmental conditions, growth was steady and fairly rapid. Some plants attained a height of 100 cm in 100 days. In periods of mild weather, numerous squares⁵ developed, several of which opened to form the cotton flower. Most of the bolls were shed within two weeks after the flower⁶ appeared. In two instances, however, bolls set and developed normally. Although they were small, the seeds were fertile and the lint mature.

The cotton plant may thus be grown successfully under the artificial régime described. Conditions were far from optimal as indicated by the large numbers of shed squares and an abnormally long time required for maturation of the bolls. With a more favorable environment, the method might be used advantageously to study the basic and optimum requirements of the cotton plant. It would be particularly useful in deter-

³ S. R. Hoover and F. E. Allison, *Trans. 3rd Internatl. Cong. Soil Sci.*, 1: 158, 1935.

⁴ A. I. Virtanen and T. Laine, *Enzymologia*, 3: 266, 1937.

¹ The term "psammoponies" to designate sand culture has been introduced by W. A. Hamor, *Ind. Eng. Chem., News Edition*, 17: 1, 1939.

² 20-30 mesh standard sand.

³ C. Ellis and M. W. Swaney, "Soilless Growth of Plants," Reinhold Publishing Co., New York, 1938.

⁴ The ferric chloride is made up in a separate solution and added to the medium just before use.

⁵ Cotton flower buds.

⁶ The flowers last 24 hours.

mining the role of trace elements in growth and fruiting.⁷ The optimal ratios of essential elements, such as N:P, could be investigated. Information on the cause of shedding might be obtained.⁸ The technic also can be used in studies of plant metabolism.⁹

Thus, this procedure, as a useful method in determining the physiological requirements of the cotton plant, may ultimately be helpful in improving the yield of lint and seed. In the present plight of cotton agriculture, any means for reducing the cost of raising cotton merits especial attention. Lower production expenses, and hence economically sound decreases in price, would stimulate domestic uses and the export trade.¹⁰

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PHYSICO-CHEMICAL PROPERTIES OF THE ROUS CHICKEN TUMOR AGENT¹

THE agent causing Rous chicken tumor I may be separated from cell-free tumor extracts by high-speed centrifugation (Ledingham and Gye, McIntosh, Elford and Andrewes, Amies, Claude, Pollard). The purified material serving for the present experiments was prepared essentially according to Claude² with the aid of an air-driven ultracentrifuge.³ The nitrogen content was 8.9 per cent. The material is precipitated from its strongly opalescent solutions by half-saturation with ammonium sulfate and by protamine. It contains thymonucleic acid. Even after four sedimentations in the high-speed centrifuge the material gives a positive hemochromogen reaction. Amounts of 10^{-7} to 10^{-9} gm produced typical sarcomas in chickens. *In vitro* the material exhibited cytochrome oxidase and catalase activity (unpublished experiments with Mr. J. L. Melnick). It remains to be decided whether these enzymatic activities are intrinsic properties of the

tumor-producing agent or whether they are due to associated substances.

The solutions of the purified agent show no appreciable flow double refraction. Their relative viscosity is 1.3 in 1 per cent. solutions after complete removal of the highly viscous mucin material present in crude tumor extracts. Measurements by the falling drop method of Barbour and Hamilton,⁴ for which we are indebted to Mr. P. H. Barbour, Jr., indicate a density of 1.23. Preparations purified by four alternate high-speed (30,000 r.p.m.) and low-speed (2,600 r.p.m.) centrifuge runs, when examined in the analytical ultracentrifuge by the light-absorption method, yielded a mean sedimentation constant $s_{20} = 550 \times 10^{-13}$ cm dynes⁻¹ sec⁻¹ (Fig. 1).

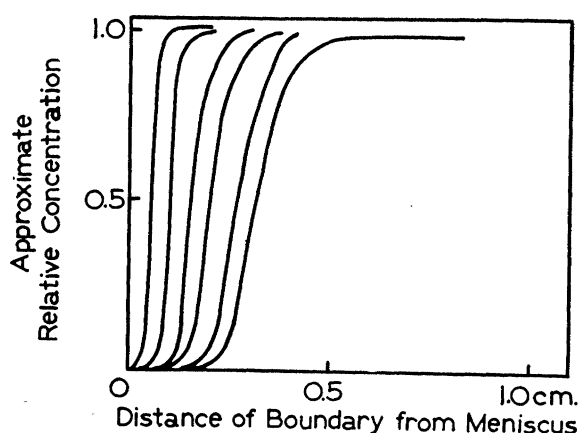


FIG. 1. Tracings of microphotometer curves obtained from sedimentation photographs of purified Rous chicken tumor I agent. Concentration of material, 1.08 per cent., in 0.005 M. phosphate, pH 7.3; mean gravitational force, 3,500 g (7,200 r.p.m.); intervals between exposures, 6 min.; length of exposure time, 5 sec. (Eastman positive film); $\lambda = 2,480-3,600$ Å (high-pressure mercury arc, bromine filter); photographic magnification, 1.5; magnification ratio during recording, 1:6.

The macromolecular material prepared from normal chick embryos according to Claude⁵ shares many physico-chemical properties with the Rous chicken tumor I agent. The average sedimentation rate of two purified preparations was found to be $s_{20} = 532 \times 10^{-13}$ cm dynes⁻¹ sec⁻¹.

While only one boundary could be observed upon sedimentation of the preparations from chicken tumor I and normal chick embryos the spreading of the boundary in the course of the runs indicated a relatively low homogeneity of the materials. The figures given above represent the sedimentation rates of the particles of average size present in populations of particles of somewhat differing dimensions. The more extensively purified preparations were found to be free

⁷ Although the unglazed pots were washed, it is possible that traces of an essential element or elements were slowly leached from them. Growth experiments in glass containers would be more conclusive.

⁸ According to Brown, "Even where there are no insects to bother, more than half the flowers that open fail to make bolls that mature." H. B. Brown, "Cotton," McGraw-Hill Book Co., New York, 1938, p. 124).

⁹ The root system is easily obtained for analysis by washing away the coarse sand in a stream of water. According to macroscopic observation, no particularly fine root hairs develop. The availability of water and nutrients apparently renders them unnecessary.

¹⁰ It is necessary to give credit to the Cotton Research Foundation (see SCIENCE, 87: 87, 1938). Since this paper was submitted we have learned that sand culture of cotton has been practiced for some time at several southern agricultural experiment stations.

¹ This investigation was aided by a grant from the Jane Coffin Childs Memorial Fund for Medical Research.

² A. Claude, *Amer. Jour. of Cancer*, 30: 742, 1937; SCIENCE, 87: 467, 1938.

³ J. W. Beams, F. W. Linke and P. Sommer, *Rev. Sci. Instr.*, 9: 248, 1938.

⁴ H. G. Barbour and W. F. Hamilton, *Jour. Biol. Chem.*, 69: 625, 1926.

⁵ A. Claude, *Proc. Soc. Exp. Biol. and Med.*, 39: 398, 1938.