set kept in the dark. Since it took more than 8 weeks for root development, many of the shoots were already turning brown. To overcome this browning and also to shorten the time required for root formation, healthy shoots from these bottles were transferred to bottles containing White's basal medium without any growth substances. Roots appeared at the end of each such shoot within 10 days (Fig. 2*D*). In this manner hundreds of tobacco plantlets with roots and shoots were obtained in vitro.

Finally these plantlets, both those grown directly from MS medium and those with roots produced on White's medium, were transferred to sterilized soil in pots in the greenhouse. The plantlets in the pots were covered by the top of the broken culture bottle for 10 to 15 days to keep the humidity high (Fig. 2*E*). More than a dozen such plants are now growing in the greenhouse (Fig. 2, *F* and *G*). Flower buds and regular flowers have also been produced by a number of these plants (Fig. 2*H*).

It has thus been demonstrated that from a completely isolated vegetative cell (single) of tobacco a whole plant capable of producing flowers can be obtained under controlled nutritional and physiological conditions. This demonstration confirms further the totipotency of single cells (at least in tobacco); such cells can grow, divide, and produce a colony of cells that can be induced to give rise to whole plants.

VIMLA VASIL
A. C. HILDEBRANDT
Department of Plant Pathology, University of Wisconsin, Madison

References and Notes

1. F. C. Steward, M. O. Mapes, K. Mears, *Am. J. Botany* **45**, 705 (1958).
2. J. Reinert, *Planta* **53**, 318 (1959); W. Halperin and D. F. Wetherell, *Am. J. Botany* **51**, 274 (1964); ———, *Science* **147**, 76 (1964); H. Kato and M. Takeuchi, *Plant Cell Physiol. Tokyo* **4**, 243 (1963); I. K. Vasil, A. C. Hildebrandt, A. J. Riker, *Science* **146**, 76 (1964).
3. V. Vasil and A. C. Hildebrandt, *Science* **147**, 1454 (1965).
4. A. C. Hildebrandt, in *Modernen Methoden der Pflanzenanalyse*, M. V. Tracey and H. F. Linskens, Eds. (Springer, Berlin, 1963), vol. 5, p. 383.
5. T. Murashige and F. Skoog, *Physiol. Plantarum* **15**, 473 (1962).
6. I. K. Vasil and A. C. Hildebrandt, in preparation.
7. F. Skoog, *Am. J. Botany* **31**, 19 (1944); F. Skoog and C. Tsui, *ibid.* **35**, 782 (1948); P. R. White, *Bull. Torrey Botan. Club* **66**, 507 (1939).
8. Work supported by AEC contract AT(11-1)-1304, by the American Cancer Society, by NIH (Project CAO 5860), and by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation. We are indebted to E. H. Herrling for assistance with the illustrations.

9 August 1965

Pituitary Gland: Enzymic Formation of Methanol from S-Adenosylmethionine

Abstract. *An enzyme has been found that can transform* S-*adenosylmethionine to methanol and* S-*adenosylhomocysteine. This enzyme is highly localized in the pituitary gland of several mammalian species.*

Methyl-*S*-adenosylmethionine, labeled with C^{14}, has been found to be useful for assaying methyltransferase enzymes and detecting new methyltransferases. In measuring methyltransferase activity, C^{14}-methyl-*S*-adenosylmethionine is incubated with enzyme and substrate, the C^{14}-methylated product formed by the enzyme is extracted into a suitable solvent and the radioactivity is measured (*1*). The use of C^{14}-methyl-*S*-adenosylmethionine made it possible to demonstrate a new catecholamine, *N*-methyladrenaline, in mammalian adrenal gland (*2*) and the formation of a wide variety of unusual *O*-methylated catechols (*3*). In the search for new transferase enzymes, C^{14}-methyl-*S*-adenosylmethionine is incubated with tissue extracts, and the products formed from normally occurring substrates are identified. One such enzyme found was a nonspecific enzyme in rabbit lung that *N*-methylates serotonin, nornicotine, normorphine, and numerous other amines (*4*). We wish to describe a new enzyme that forms methanol from *S*-adenosylmethionine.

In a study of the tissue distribution of the adrenaline-forming enzyme (phenylethanalamine *N*-methyltransferase) we observed that the incubation of pituitary glands with C^{14}-methyl-*S*-adenosylmethionine resulted in the formation of a radioactive product that was extractable into organic solvents such as isoamyl alcohol. When the solvent was concentrated in a vacuum for identification by paper chromatography, a considerable portion of the radioactivity was lost, suggesting the presence of a volatile radioactive metabolite. To further characterize the enzyme and the metabolic product, C^{14}-methyl-*S*-adenosylmethionine was incubated with various subcellular fractions of the bovine pituitary gland (Table 1). After incubation the mixture was extracted with isoamyl alcohol and radioactivity was measured in portions of the extract before and after evaporation in a stream of warm air. After evaporation it was apparent that considerable amounts of the radioactivity had been lost. Heating the pituitary extract at 100°C destroyed all activity and thus demonstrated that the volatile product was formed enzymatically. The ability to form the volatile metabolite resided mainly in the soluble supernatant fraction. The nonvolatile metabolite was identified as methylhistamine by paper chromatography (*5*). This identification was not unexpected, for large amounts of endogenous histamine and histamine methyltransferase (*6*) are present in the posterior pituitary gland.

The enzyme that forms the volatile metabolic product was purified about 13-fold from bovine posterior pituitary by $(NH_4)_2SO_4$ precipitation, $Ca_3(PO_4)_2$ gel (negative adsorption), and alumina-C_γ gel chromatography. After incubation of a partially purified enzyme only the volatile metabolic product was detected.

One volatile metabolic product that might arise from *S*-adenosylmethionine is methanol. Its enzymic formation was examined as follows: After incubation of the partially purified enzyme with C^{14}-methyl-*S*-adenosylmethionine at *p*H

Table 1. Enzymatic formation of a volatile metabolite from C^{14}-methyl-*S*-adenosylmethionine in the bovine pituitary gland. Posterior pituitaries were homogenized with 10 volumes of cold distilled water, and the supernatant fraction was separated from the particulate by centrifugation at 80,000*g*. Various subcellular fractions obtained from 10 mg of pituitary gland were incubated with 37 nmole of C^{14}-methyl-*S*-adenosylmethionine (activity, 10^4 count/min) in 50 μl of 0.5*M* phosphate buffer (*p*H 7.9) in a final volume of 200 μl in a 15-ml, glass-stoppered centrifuge tube. After 60 minutes of incubation at 37°C, 0.5 ml of borate buffer (*p*H 10) and 6 ml of isoamyl alcohol were added and the mixture was shaken for 5 minutes. After centrifugation, 2-ml portions of isoamyl extract were transferred to vials. To one vial, 2 ml of ethanol and 10 ml of phosphor were added and the radioactivity was measured. The isoamyl alcohol in the other vial was evaporated to dryness is a stream of warm air, and radioactivity was measured after addition of 2 ml of ethanol, 2 ml of isoamyl alcohol, and 10 ml of phosphor. The radioactivity found in a 2-ml portion of isoamyl alcohol is reported.

Preparation	Radioactivity (count/min)	
	Before evaporation	After evaporation
Soluble supernatant fraction	1900	1280
Particulate fraction	49	25
Soluble supernatant fraction heated at 100°C (3 min)	58	50
$(NH_4)_2SO_4$ fraction from precipitate of posterior pituitary at 30 to 45% saturation (0.68 mg of protein)	895	60

Table 2. Methanol-forming enzyme in the pituitary gland of various mammalian species. Soluble supernatant fractions obtained from 10 mg of pituitary from various species were incubated and assayed as described in Table 1. Results are expressed as nanomoles of methanol formed per gram of tissue.

Species	Pituitaries examined (No.)	Enzyme activity
Cattle, posterior pituitary	(5)	80
Cattle, anterior pituitary	(3)	25
Rabbit, whole pituitary	(2)	105
Rat, whole pituitary	(3)	35
Human, whole pituitary	(2)	17

7.9, 50 μl of nonradioactive methanol and 5 ml of ethyl acetate were added. The mixture was dried with sodium sulfate and the methanol was converted to 3,5-dinitrobenzoate with 3,5-dinitrobenzoyl chloride and sodium carbonate. The methyl 3,5-dinitrobenzoate was recrystallized to a constant melting point and to constant specific activity. This material showed only one peak of radioactivity on thin-layer chromatography; this peak coincided with the one shown by authentic methyl 3,5-dinitrobenzoate. These observations were taken as evidence that the volatile product formed enzymatically from pituitary extracts and C^{14}-methyl-*S*-adenosylmethionine was methanol.

The other product formed from this reaction should be *S*-adenosylhomocysteine. To establish this, *S*-adenosylmethionine-2-C^{14} (*7*) was incubated with a partially purified enzyme preparation. After incubation the enzyme mixture was applied directly to Whatman No. 1 paper and chromatographed. The radioactive metabolite formed corresponded in R_F value on paper chromatograms to authentic *S*-adenosylhomocysteine in two solvent systems. In parallel experiments the relative conversion of C^{14}-methyl-*S*-adenosylmethionine to methanol and *S*-adenosylmethionine-2-C^{14} to *S*-adenosylhomocysteine was found to be stoichiometric. These results demonstrate that the enzyme in the pituitary gland carries out the following reaction:

S-adenosylmethionine + H_2O → *S*-adenosylhomocysteine + CH_3OH

This reaction proceeds by methylation of water or by hydrolysis of *S*-adenosylmethionine.

The methanol-forming enzyme was assayed in pituitary gland (Table 2) and other tissues of various species by the procedure described in Table 1. In cattle the anterior pituitary had about 25 percent of the activity of the posterior pituitary. All mammalian pituitaries examined (human, rat, rabbit) contained enzyme activity. The methanol-forming enzyme was also examined in tissues of ten organs of the rabbit. Negligible activity was present in these tissues; the small amounts of methanol found might have resulted from further metabolism of methanol. Whole brain and skin of frog (*Rana pipiens*) contained the methanol-forming enzyme. An ectopic tumor of possible pineal or pituitary origin also contained large amounts of the methanol-forming enzyme (*8*), but no hydroxyindole *O*-methyltransferase, an enzyme found in tissues of pineal gland (*9*). This specificity of the enzyme indicates that this tumor was of pituitary origin.

Methanol is present in the normal breath of human subjects (*10*) and is believed to be formed endogenously. However, no pathway for the formation of methanol in animals has been demonstrated previously. It is possible that this alcohol might arise from the action of the methanol-forming enzyme on *S*-adenosylmethionine, in the pituitary gland. Although the role of the methanol-forming enzyme remains obscure, its localization in an organ of high physiological activity, the pituitary gland, suggests that it might have a function.

JULIUS AXELROD
JOHN DALY
National Institute of Mental Health and National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland, 20014

References and Notes

1. J. Axelrod, in *Transmethylation and Methionine Biosynthesis*, S. R. Shapiro and F. Schlenk, Eds. (Univ. of Chicago Press, Chicago, 1965), pp. 71–83.
2. J. Axelrod, *Biochim. Biophys. Acta* **45**, 614 (1960).
3. J. Daly, J. K. Inscoe, J. Axelrod, *J. Med. Chem.* **8**, 153 (1965).
4. J. Axelrod, *J. Pharmacol. Exp. Therap.* **138**, 28 (1962).
5. D. D. Brown, J. Axelrod, R. Tomchick, *Nature* **183**, 680 (1959).
6. H. M. Adam, in *Regional Neurochemistry*, S. S. Kety and J. Elkes, Eds. (Pergamon, Oxford, England, 1961), pp. 293–306; J. Axelrod, P. D. MacLean, R. W. Albers, H. Weissbach, *ibid.*, pp. 307–311.
7. We thank S. H. Mudd for supplying us with *S*-adenosylmethionine-2-C^{14}.
8. S. H. Snyder, J. Axelrod, O. Smith, unpublished observations.
9. R. J. Wurtman, J. Axelrod, R. Toch, *Nature* **204**, 1323 (1964).
10. S. P. Eriksen and A. B. Kulkarni, *Science* **141**, 639 (1963).

9 August 1965

Bone: Formation by Autoinduction

Abstract. *Wandering histiocytes, foreign body giant cells, and inflammatory connective-tissue cells are stimulated by degradation products of dead matrix to grow in and repopulate the area of an implant of decalcified bone. Histiocytes are more numerous than any other cell form and may transfer collagenolytic activity to the substrate to cause dissolution of the matrix. The process is followed immediately by new-bone formation by autoinduction in which both the inductor cells and the induced cells are derived from ingrowing cells of the host bed. The inductor cell is a descendant of a wandering histiocyte; the induced cell is a fixed histiocyte or perivascular young connective-tissue cell. Differentiation of the osteoprogenitor cell is elicited by local alterations in cell metabolic cycles that are as yet uncharacterized.*

New evidence in favor of the theory of induction can be gathered from the process of bone formation in the interior of an implant of acellular, devitalized, decalcified, bone matrix. Differing from previous demonstrations of induction systems, which produce scanty deposits and less than 30-percent positive results (*1*), decalcified bone yields new bone in an amount proportional to the volume of the implant; the percentage of positive experimental results is as high as over 90 percent. The results of some 70 experiments on approximately 300 animals are summarized below to introduce a hypothesis of postfetal osteogenesis by autoinduction (*2*).

Long bones, excised from adult rabbits and other laboratory animals, were cut in lengths of 1 to 2 cm and decalcified, unfixed, in 0.6*N* HCl. Samples of human cortical bone obtained from accident cases, excised under aseptic conditions at autopsy, were lyophilized and decalcified similarly in sterile solutions of 0.6*N* HCl for a period of 5 days. The acid was removed by prolonged washing in sterile 0.15*M* NaCl. The chemical composition of HCl-decalcified bone matrix, in millimoles per liter, per kilogram,