Table 1. Encephalitogenic activity of bovine basic proteins.

Amount tested (μg)	Day of onset*	Disease index†
	S-7 total fraction‡	
20	13	9.1
5	18	6.0
1		2.6
	S-7 major protein‡	
20	12	8.4
5	15	7.8
1	20	4.4
	Component IV‡	
20	14	8.9
5	18	7.4
1		2.2

* Day on which the third of the group of five guinea pigs showed definite clinical signs of EAE. † Combined clinico-pathologic index of severity of EAE (average of five animals), the maximum being ten (*6*). ‡ Prepared by Martenson and LeBaron (*5*).

homogenate. This interaction may result in cleavage of the protein-lipid bonds (*2*).

The major portion of the encephalitogenic activity of whole CNS tissue is obtained by acid extraction of tissue which has been previously extracted with chloroform-methanol. These acid extracts may be further purified, yielding highly encephalitogenic as well as nonencephalitogenic basic proteins (*3, 4*). In addition, small amounts of acidic and neutral protein contaminants have been identified immunochemically. These are nonencephalitogenic.

Martenson and LeBaron (*5*) have reported purification of a group of basic proteins isolated in the course of an investigation of protein-bound phosphoinositides in the chloroform-methanol insoluble residue of CNS white matter. Chemical similarities between two of these basic proteins, which they call "major S-7 component" and "component IV," and the encephalitogenic myelin basic protein suggested that these preparations were identical with the encephalitogen. Consequently arrangements were made to test the unknown preparations under the same conditions used for bioassay of the previously reported encephalitogenic proteins (*6*). Equally susceptible inbred strain 13 guinea pigs were used instead of the random-bred NIH animals. The fractions were prepared from bovine white matter that had been extracted with chloroform and methanol. They were S-7 (that part of the acid extract soluble at *p*H 7), the major protein component of S-7, and component IV which is a subfraction of the material insoluble at *p*H 7. When 0.02 mg of each fraction was tested, maximum EAE resulted in all of the guinea pigs injected. Each fraction was again tested at 0.001 and 0.005 mg (Table 1). All three are encephalitogenic with 50 percent effective dose (ED_{50}) of about 2 μg. The major component of fraction S-7 probably accounts for all of its encephalitogenic activity. Component IV, possessing approximately the same activity as the major S-7 component, appears to be closely related to the latter even though the two were originally obtained from two fractions of the acid extract with different solubilities.

The major protein of the fraction soluble at *p*H 7, as well as component IV of the fraction insoluble at *p*H 7, can thus be added to the increasing list of encephalitogenic proteins reported (*2, 4, 7*). It is unlikely that all of these preparations are identical. Quite probably they are closely related, at least as indicated by their chemical and biological properties. Gel filtration on Biogel P-10 of component IV and of the major S-7 component indicates that they are similar in size (35,000 to 40,000 molecular weight) and that they fall into the category of "large" encephalitogens characteristically obtained from CNS tissue which had been previously extracted with chloroform-methanol in contrast to "small" encephalitogens (about 10,000 molecular weight) frequently obtained from tissue that had previously been extracted with acetone.

These findings are all the more interesting in that the same purified protein was obtained in two independent studies. In one, bioassay was the criterion of progressive purification. In the other, chemical analysis alone was the criterion. Furthermore, in both cases, we have been impressed by the relative abundance of this protein in CNS tissue and its remarkable stability in the presence of chloroform-methanol and of relatively strong acid.

The bioassays strengthen the opinion of Martenson and LeBaron (*5*) that the major component of the S-7 fraction is the same protein as component IV. Encephalitogenic activity in more than one fraction has also been observed during isolation of the encephalitogen (*8*). A possible explanation is that there is an equilibrium between free and combined protein, the bound forms being less soluble than free protein at neutral *p*H. If so, solution of the insoluble complex in dilute acetic acid and chromatography on carboxymethyl cellulose appears to cause dissociation of the complex. Free protein is then isolated as component IV.

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9. Supported by research grant 239 from the National Multiple Sclerosis Society, grant NB-03147 from PHS, and by fellowship supply grants NB-00572 and NB-00130 from PHS.

12 November 1965

Intracellular Localization of Growth Hormones in Plants

Abstract. *Autoradiographic studies of* Allium cernuum *and* Vicia faba *root-tip cells treated with indoleacetic acid-methyl-C^{14} or 2,4-dichlorophenoxyacetic acid-carboxyl-C^{14} revealed nuclear and cytoplasmic labeling of the cells. The cytoplasmic labeling decreased with time after the removal of the labeled auxin, but nuclear and chromosomal labeling was retained for at least 120 hours.*

There is evidence that auxin-induced growth in various plant tissues is accompanied by changes in the cell wall and cytoplasm, and in nucleic acid metabolism (*1*). However, it is unknown which of these is the direct effect of auxin action. It has been shown by autoradiography that the plant growth inhibitor maleic hydrazide-C^{14} was localized in the nuclei of root-tip cells for a short time interval after treatment. Subsequent time-course experiments indicated movement of the inhib-

itor into the nucleolus and still later from the nucleolus to the cytoplasm (*2*). We report evidence, detected by autoradiographic techniques, that exogenously supplied indoleacetic acid-methyl-C^{14} (IAA-C^{14}) and 2,4-dichlorophenoxyacetic acid-carboxyl-C^{14} (2,4-D-C^{14}) are localized in both the cytoplasm and the nuclei of root-tip cells.

The techniques used were in general the same as those of Callaghan and Grun (*2*). *Allium cernuum* plants were grown in greenhouse pots. Root tips, 1 cm long, of rapidly growing plants were excised and placed in half-strength Hoagland's solution containing 1.0 μg/ml of IAA-C^{14} (1.5 mc/mmole). At the end of a 6-hour incubation period the samples were fixed for 10 minutes in a mixture of glacial acetic acid, 100 percent ethyl alcohol, chloroform, and formalin (10 : 2 : 2 : 1) (*3*). The tissues were then washed with distilled water and incubated with 5 percent pectinase in 1 percent peptone solution at 37°C for 1 hour. After the pectinase treatment the root tips were washed, and tissues of the first 5 mm of the tip were squashed in one drop of 45 percent aqueous solution of lacto-propionate-orcein (*3*).

Autoradiography was carried out with Kodak AR.10 autoradiographic stripping film. After the film was mounted over the root-tip tissue and allowed to dry, the slides were stored, for an exposure time of 4 weeks to 6 months, at 2°C in light-tight boxes that contained a dessicant.

After the results of these experiments were obtained a series of time-course experiments were conducted to follow the possible movement of intracellular label. In a typical experiment *Vicia faba* seeds were germinated on white sand at 20° to 22°C and transferred into 50-ml test tubes containing half-strength Hoagland's solution when the primary root of each plant was about 8 cm long. When the plants were 4 weeks old their secondary roots were immersed in 100 ml of half-strength Hoagland's solution containing 0.75 μg/ml of 2,4-D-C^{14} (22.3 mc/mmole) for 30 minutes or 1 hour. The plants were then thoroughly rinsed with distilled water and returned to the culture solution without 2,4-D-C^{14}. Root-tip samples were harvested at 0, 24, 48, 72, 96, and 120 hours after the plants were removed from 2,4-D-C^{14}. All the samples were fixed, squashed, and prepared for autoradiography as described for the experiment with IAA-C^{14}.

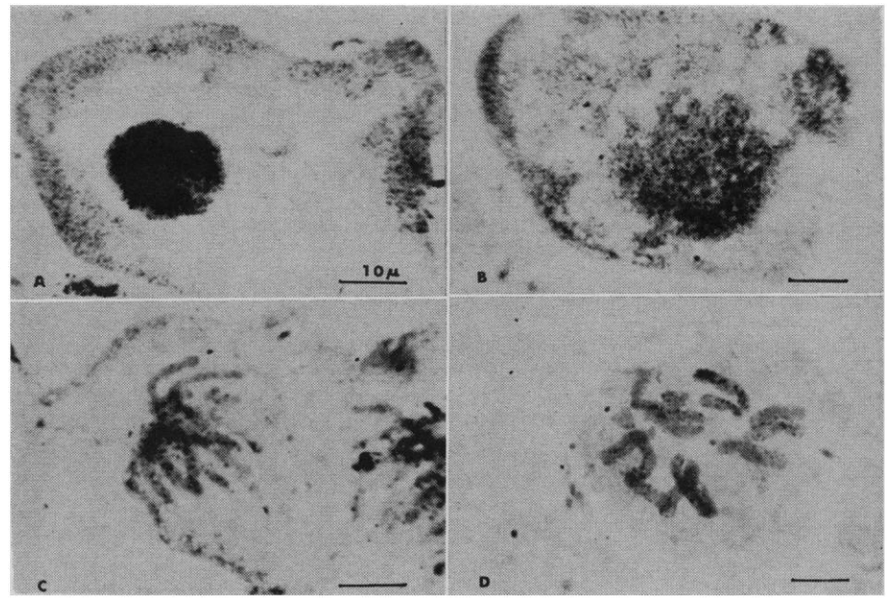

Fig. 1. Autoradiographs of auxin-treated cells. (*A*) *Allium cernuum* root-tip cell fixed after a 6-hour treatment with indoleacetic acid-methyl-C^{14}, showing nuclear and cytoplasmic labeling. (*B*) *Vicia faba* root cell fixed after treatment for 1 hour with 2,4-dichlorophenoxyacetic acid-carboxyl-C^{14} (2,4-D-C^{14}). Note heavy cytoplasmic and nuclear labeling. (*C*) Same treatment but fixed 72 hours after the removal of 2,4-D-C^{14}. The label is concentrated on the anaphase chromosomes. (*D*) Chromosomal labeling retained in cell fixed 120 hours after removal from 2,4-D-C^{14}.

In tissue treated with IAA-C^{14} there was cytoplasmic and pronounced nuclear labeling in most of the meristematic cells (Fig. 1*A*). Results with tissue treated with 2,4-D-C^{14} showed that both cytoplasm and nuclei of cells fixed at the end of incubation were labeled (Fig. 1*B*). The cytoplasmic labeling decreased with the time after incubation and was not observed in the 120-hour preparations (Fig. 1, *C* and *D*); however, nuclear labeling was found at all time intervals after the removal of the 2,4-D-C^{14} (Fig. 1, *B*, *C*, and *D*). In mitotic cells the nuclear labeling was localized on the chromosomes (Fig. 1, *C* and *D*).

In *A. cernum*, the binding of label from IAA-C^{14} or 2,4-D-C^{14} into the cytoplasm or nucleus occurs mainly in undifferentiated young cells or along the thin layer of slime material in some phloem cells. This suggests that cell type and the physiological state of the cell are important factors in incorporation of label from 2,4-D or IAA into the cellular components.

In our experiments, the cytoplasmic labeling was observed only in cells fixed shortly after the removal of labeled auxins, but the nuclear and chromosomal labeling remained at least for 120 hours. At no time was pronounced nucleolar labeling observed. These results suggest that there may be two kinds of binding sites, namely, nuclear and cytoplasmic, for auxin-type growth hormones in meristematic root-tip cells. Auxins cause an increase in the synthesis of a specific RNA fraction (*4*), and inhibitors of protein and RNA synthesis inhibit auxin-stimulated growth of plant tissue (*5*). A labeled 4*S* RNA fraction has been obtained from pea sections following exposure to IAA-C^{14} (*6*). The binding of β-naphthaleneacetic acid-1-C^{14} to isolated coconut nuclei associated with release of nuclear RNA has also been reported (*7*). RNA extracted from Jerusalem artichoke, *Saccharomyces ellipsoideus*, and *Avena* coleoptiles has been reported to be effective in increasing expansion of excised discs of artichoke tuber treated with 2,4-D or 2,4-D plus kinetin (*8*). Hence, it is quite possible that the labeling revealed by our autoradiographic studies is at least in part associated with RNA.

It is also apparent that some chromosomal component is responsible for the nuclear binding of label from 2,4-D or IAA, since the mitotic chromosomes were labeled. It is impossible in this study to conclude whether the chromosomal labeling is due to binding by RNA, DNA, histone, or nonhistone proteins of the chromosomes (*9*). Although it has not been determined whether the radioactivity bound to the

nuclear material and the cytoplasm is undegraded IAA-C^{14} or 2,4-D-C^{14}, the fact that both give the same labeling pattern may suggest that degradation is not involved.

Our positive results could be regarded as due to some nonselective binding of 2,4-D-C^{14} or IAA-C^{14} to the cellular components or to some artifact during preparation. However, these results seem to indicate some physiological significance, since only certain meristematic cells acquire most of the label, and control preparations of root tips not exposed to radioactive IAA or 2,4-D cause no detectable labeling above the background on the film.

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17 December 1965

Phenylalanine: Transplacental Concentrations in Rhesus Monkeys

Abstract. *Amino acids are actively transported across the mammalian placenta, with concentrations in fetal blood being higher than those in the maternal circulation. Elevated concentrations of phenylalanine were induced by dietary means in the blood of pregnant rhesus monkeys, and the active transport mechanism was evident at both normal and elevated concentrations. A normal placental process may thus magnify a maternal biochemical abnormality and produce a more profound disturbance in the fetus.*

The mechanism responsible for mental retardation in phenylketonuria is unknown, but is associated with high concentration of phenylalanine in the blood. Recent reports indicate that infants born to mothers having phenylketonuria may be retarded without measurable abnormality in phenylalanine metabolism (*1*); they suggest that an elevated concentration of phenylalanine in the maternal blood may cross the placenta and in some way damage the fetal brain. We have evaluated this hypothesis by measuring maternal and fetal blood concentrations of phenylalanine (*2*) in full-term pregnancies in the rhesus monkey.

Female monkeys were mated and the dates of conception were established; all were individually caged and records were kept of dietary intake, weight, and clinical status. In eight control pregnancies the animals were fed commercial chow, milk, vitamins, and fruit. Infants were separated from mothers from the moment of birth. From simultaneous blood specimens from the mother and from the umbilical cord of her infant were determined the normal free amino acid concentrations (*3*); two sets of maternal and cord specimens were obtained at the time of elective cesarean section.

In six other pregnancies the maternal diet was supplemented with excess L-phenylalanine in amounts ranging from 0.2 to 1.4 g day^{-1} per kilogram of body weight, which was added to commercial milk which, with vitamins and fruit, provided the total nutrition. In order to maintain a constant elevation of serum phenylalanine it was necessary to feed the supplemented milk at 4-hour intervals (*4*). Serum phenylalanine was determined at biweekly intervals during the pregnancy, each blood specimen being obtained 4 hours after the previous dietary intake. After birth, maternal and cord-blood specimens were analyzed for all free amino acids. In two pregnancies, cord blood was unobtainable and a specimen of the infant's peripheral blood was collected within 5 minutes of birth.

In control pregnancies the cord:maternal ratio (C:M) was greater than unity for all amino acids. The mean serum concentrations of phenylalanine in mother and cord were 1.26 and 1.67 mg/100 ml, respectively; the mean C:M for phenylalanine was 1.49 (Table 1).

Females fed the phenylalanine-supplemented diet had mean concentrations of phenylalanine in serum, during pregnancy, ranging from 14.7 to 40.4 mg/100 ml. One pregnancy resulted in death of the fetus because of its abnormal position at the time of birth. A second mother refused to eat and became dehydrated during the last 2 days of pregnancy; both maternal and cord serums in this pair revealed generalized hyperaminoacidemia.

The four other females whose diets were phenylalanine-supplemented produced clinically normal infants at full-term pregnancy. Signs of nutritional or neurologic damage were not detected in any infant. The concentration of phenylalanine in the serums of mothers

Table 1. Transplacental concentrations of phenylalanine and tyrosine in serums and cord:maternal ratios (C:M) in four mother-fetus couples.

Phenylalanine (mg/100 ml)			Tyrosine (mg/100 ml)		
Cord	Mother	C:M	Cord	Mother	C:M
Pregnant mother's diet supplemented with phenylalanine					
45.3	22.0	2.06	11.2	7.5	1.49
43.1	11.8	3.65	13.7	4.6	2.98
43.7	27.2	1.61	11.9	6.3	1.61
12.88	6.99	1.84	4.09	2.41	1.70
Eight controls (average)					
1.68 ± 0.28	1.26 ± 0.49	1.49 ± 0.58	1.46 ± 0.33	0.93 ± 0.29	1.66 ± 0.56