

activity of the isolated pentose was 70 and 80% of the expected maximum value in the two experiments mentioned above. This indicates that dilution of the synthesized pentose occurred, most likely, at a stage subsequent to the interaction of erythrose phosphate and formaldehyde. The evidence indicates a direct condensation of erythrose phosphate with formaldehyde as the first step in this biosynthesis. The phosphate bond in the pentose phosphate is stable to acid hydrolysis (1 *N* HCl at 100° for 30 minutes releases 5–10% of the organic phosphorus), thus excluding ribose 1-phosphate and favoring ribose 5-phosphate. No addition of any cofactor was required by the enzyme systems used.

TABLE I

Unlabeled	Substrates Labeled	Spec. act. of substr. ^a	Spec. act. of pentose	Theoret. spec. act. ^b
Formald.	Erythrul. phosph.	13,300	9,300	13,300
.....	Erythrul. phosph.	13,300	<100	None
.....	Formaldehyde	30,000	Zero	None
.....	{ Formaldehyde + Erythrul. phosph.	{ 30,000 13,300	34,760	43,300

^a All specific activities are expressed as c.p.m. per micro-mole. ^b Assuming a utilization of one mole each of formaldehyde and erythrose phosphate per mole of pentose formed.

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RECEIVED JANUARY 14, 1955

KINETIN, A CELL DIVISION FACTOR FROM DEOXY-RIBONUCLEIC ACID¹

Sir:

Haberlandt's early concept of a specific cell division hormone (wound hormone) in plants has been strengthened gradually by evidence both for the specific need and for its satisfaction by extracts or substances of natural origin. For example, a factor required for cell division is practically lacking in pith but is present in limited amounts in vascular stem tissue and leaves of tobacco and in various plant products.² Yeast is a rich source, the further exploration of which now has led to deoxyribonucleic acid (DNA) as the starting material³ for the isolation of a physiologically highly active chemical. The name *kinetin* is suggested for this substance.

For the bioassay tobacco "wound" callus tissue was used. It was obtained from stem segments grown on White's agar medium with 2 mg./l. indoleacetic acid (IAA) added. This tissue cultured on the same medium will undergo cell enlargement and limited increase in weight in response to the added IAA, but is incapable of cell division and continued growth unless a cell division factor is supplied. Rapid (5–10 day) assays of kinetin therefore are based on visual estimates of increased cell division

(1) This work was supported in part by research grants from the American Cancer Society, the National Science Foundation, and the Wisconsin Alumni Research Foundation.

(2) J. R. Jablonski and F. Skoog, *Physiol. Plantarum*, **7**, 16 (1954).

(3) In *l'Année biologique*, **30**, 412 (1954).

activity as well as continuous growth of these cultures. The validity of the test has been established by cytological examinations and by determinations of weight increases.

For example, in one experiment determinations after six weeks showed that callus pieces had an average fresh weight of 1340 mg. in the presence of 100 µg./l. of kinetin, as compared with 1320 mg. in its absence. The corresponding dry weights were 101 and 73 mg. per piece, but the ratio of cell numbers treated/controls was 31/1 as estimated from counts in sections.

The first sample of DNA tested (a 4-year old preparation from herring sperm) was active, but fresh preparations from this source were inactive. However, on autoclaving water slurries of new DNA (pH 4.3) at 15 lb. for 30 min., they became extremely active. DNA from calf thymus behaved similarly. After extracting from aqueous solution (pH 6.8) with *n*-butanol and removing the alcohol, the active material in water was put on a cation exchange resin (Dowex 50) column and was eluted with 1.5 *N* HCl. The kinetin came off slowly and was detected by optical density readings at 271 mµ. The pooled active fractions were run through a similar column and after washing with water, the kinetin was readily eluted with 1 *N* NH₄OH. A band of crystalline material moving down the column was collected with as little dilution as possible. The precipitate from cooled solutions of this eluate was recrystallized from water, and then from absolute ethanol to give colorless platelets which sublimed at 220° (hot stage) m.p. 265–266° (sealed tube). The yield was about 30 mg. from 100 g. of DNA. The substance contained nitrogen, but no sulfur, phosphorus or halogens. Calcd. for C₁₀H₉N₅O: C, 55.81; H, 4.22; N, 32.55; mol. wt., 215.2. Found⁴ (on separate preparations): C, 56.06, 56.13; H, 4.09, 4.16; N, 32.55, 32.58. Electrometric titration⁵ in 1:1 ethanol:water showed *pK*_{a1} 2.7 ± 0.2, *pK*_{a2} 9.9 ± 0.2, n.e. (based on *pK*_{a2}) 223 ± 15. The ultraviolet spectrum⁶ showed a single band, λ_{max}^{EtOH} 268 mµ, ε 18,650, λ_{min}^{EtOH} 233 mµ, ε 3200. The infrared spectrum⁶ measured in a KBr pellet showed strong bands at 3.20, 3.30, 3.40, 3.59, 6.20, 6.30, 6.88, 7.12, 7.50, 7.64, 8.01, 8.72, 9.91, 10.7, 11.0, 11.2, and 13.3 µ. The biological activity was not destroyed by autoclaving aqueous solutions of kinetin at pH 0.5 or 12.0.

Kinetin is slightly soluble in cold water, ethanol, or methanol, freely soluble in dilute aqueous HCl or NaOH, and can be extracted from neutral aqueous solutions by shaking with ether. It is physiologically active at very great dilutions but only in the presence of auxin (added IAA). The lowest concentration tested, 0.01 p.p.m., was effective. The supply of kinetin, like that of auxin (IAA), must be renewed with each successive subculturing.

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RECEIVED JANUARY 28, 1955

(4) Microanalyses by Micro-Tech Laboratories, Skokie, Illinois.

(5) Kindly carried out by N. S. Ling and R. M. Bock.

(6) Kindly determined by S. M. Aronovic.