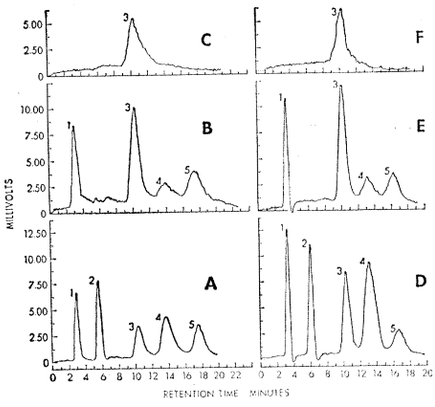

Fig. 1. Gas-coulometric chromatograms of a standard solution (*A* and *D*), and portions of a single extract of human fat (*B*, *C* and *E*, *F*) passed through two columns. First column (*A* to *C*): 1.22 m by 0.635 cm outer diameter, packed with 5 percent Dow oil grease on 30/60 mesh acid-washed Chromasorb P, at a column temperature of 182°C, nitrogen being used as a carrier gas at a flow rate of 88 cm³/min. Second column (*D* to *F*): aluminum, 1.83 m by 0.635 cm outer diameter, packed with 20 percent high-vacuum silicone grease on 30/60 mesh acid-washed Chromasorb P, at a column temperature of 215°C, nitrogen being used as a carrier gas at a flow rate of 240 cm³/min. The standard solution produced five peaks in chromatograms *A* and *D* representing: 1, benzene hexachloride; 2, aldrin; 3, dieldrin plus *p,p'*-DDE; 4, *o,p'*-DDT plus *p,p'*-DDD; and 5, *p,p'*-DDT. Chromatograms *B* and *E* were obtained from samples of the first 100 ml of benzene eluate from a florisil column, while chromatograms *C* and *F* were from samples of the final (acetone-acetonitrile) eluate from the same column. Peak No. 3 represents DDE in chromatograms *B* and *E* and dieldrin in chromatograms *C* and *F*.

viduals showing both isomers in their fat and in two others who showed no *p,p'* isomer, with an average of 1.54 times as much *o,p'*-DDT as *p,p'*DDT in the 18 people and 1.31 times as much in the entire group.

Standards were run by Schechter-Haller analysis in which the ratios of *o,p'*- to *p,p'*-DDT were varied while holding DDE constant and then applying the two-color equation for technical DDT and *p,p'*-DDE. When the ratios of *o,p'*- to *p,p'*-DDT were 20 : 80, 50 : 50, and 80 : 20, respectively, the recoveries of DDE and DDT were 97 and 109 percent, 118 and 94 percent, and 131 and 72 percent, respectively. These recoveries were obtained when the amount of DDE was aproximately equal to the sum of the *o,p'*- and *p,p'*-DDT isomers. When DDE was decreased to one-half the sum of the *o,p'*- and *p,p'*-DDT isomers, and the isomer ratio was 80 : 20, the recoveries of DDE and DDT were 188 percent and 71 percent, respectively. This means that for fat samples in which *o,p'*-DDT is increased relative to *p,p'*-DDT (in comparison to technical grade DDT used in establishing the two-color equation), results obtained by the Schechter-Haller method reflect higher DDE and lower DDT than is actually present.

The average total DDT-derived material expressed as DDT (6.69 ± 1.02 ppm) found by gas chromatography was only 62 percent of that found by the Schechter-Haller method for the same eluates (Table 1) and the difference is significant ($p = < .05$). Since recovery of standards by gas chromatography was good (95 to 100 percent), the differences obtained by the two methods when the same eluates were used must have been due to one or both of the following reasons: (i) Schechter-Haller calculation with equations based on a 20 : 80 storage ratio of *o,p'*- to *p,p'*-DDT, and (ii) variations in Schechter-Haller blanks.

By gas chromatography, DDE calculated as DDT averaged 62 percent of the total DDT-derived materials and agrees well with the 58 percent average reported for DDE by Hayes *et al.* (*2*).

Aldrin, heptachlor, heptachlor epoxide, and methoxychlor were not detected in any of the samples, but would have been detected had they been present in concentrations similar to those of BHC, dieldrin, and DDT-derived compounds.

WILLIAM E. DALE
Communicable Disease Center,
U.S. Public Health Service,
Atlanta, Georgia

GRIFFITH E. QUINBY
Communicable Disease Center,
U.S. Public Health Service,
Wenatchee, Washington

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Partial Synchronization of Nuclear Divisions in Root Meristems with 5-Aminouracil

Abstract. *Root tip cells of* Vicia faba *were partially synchronized in nuclear stages by treatment for 24 hours with 700 parts of 5-aminouracil per million. All division was suppressed by the analog treatment, and a peak in division stages (up to 62.5 percent) was reached 14 hours after removal from the aminouracil. Populations of partially synchronized cells can be useful in experiments designed to study various intracellular reactions or responses at different stages in the nuclear cycle.*

Partial synchrony of nuclear divisions has been achieved in root tip meristems of *Vicia faba* (broad bean) by treatment with 5-amino-2,4-dioxypyrimidine or 5-aminouracil (5AU).

Two types of experiments were performed. The first was designed to determine 5AU concentrations that would synchronize mitoses by stopping cell divisions and then, when treatment was terminated, would result in synchronous resumption of mitoses giving an eventual maximum proportion of dividing nuclear stages. The purpose of the second set of experiments, in which autoradiography with tritiated thymidine was used, was to study DNA synthesis after treatment with 5AU.

Roots of *Vicia faba* were grown in half-strength Hoagland's nutrient solution, treated with 5AU, washed in water, and then transferred back to nutrient solution for further growth. All treatment and recovery solutions were aerated and maintained at 20°C in the dark. All 5AU treatments were of 24 hours' duration, and the solutions contained 700 ppm of the pyrimidine base in deionized water. Exploratory experiments indicated that the specific time and concentration were not critical, but those used appear to be effective maxima. For the mitotic synchronization studies, summarized in Fig. 1, secondary roots were fixed in modified Carnoy's solution (3:2:1), stained with Feulgen, and squashed on slides.

For autoradiographic studies (Table

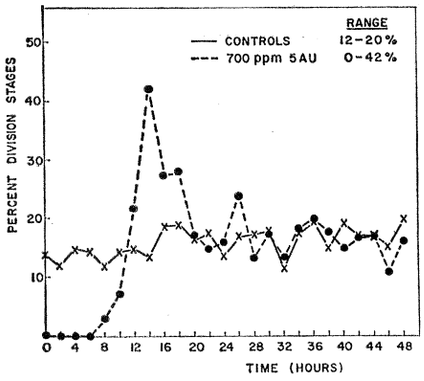

Fig. 1. Frequency of division stages in nuclei synchronized with 5-aminouracil compared with frequency in untreated controls.

1) roots were incubated with 1.5 μc of tritiated thymidine per milliliter. Secondary root tips were fixed in ethanol–acetic acid (3:1) either immediately after labeling or 14 hours after 5AU treatment, embedded in paraffin, and sectioned at 5 microns. Slides of mid-longitudinal sections were dipped in liquid nuclear track emulsion (NTB), exposed for 12 days, developed, and stained with Delafield's hematoxylin. Autoradiographic grain counts were made over nuclei in interphase and mitotic stages in the meristem.

The results of the synchronization studies with 5AU, graphed in Fig. 1, show the average percentage of nuclei in division stages. Points on the graph represent samples of three to nine root tips collected at 2-hour intervals over a 48-hour period. In the control curve, dividing stages constitute from 12 to 20 percent of the total nuclei counted. In the cells treated with 5AU no divisions were observed for 6 hours after termination of the treatment. The frequency then rose steeply to an average peak of 42 percent at 14 hours after treatment, declined steeply, and reached control levels in 20 to 22 hours. The smaller peak at 26 hours may be an artifact or it may be due to unknown events. Clearly, little synchronization is maintained through a second cycle because 20 to 22 hours after the first peak there is virtually no increase in the percentage of dividing cells above control levels.

Among the six root tips that contributed to the average peak value at 14 hours, the maximum frequency of dividing nuclei was 62.5 percent. This corresponds to the highest percentage obtained in the H^3-thymidine experiments (Table 1) and may represent a maximum synchrony achievable in this system without further improvements in technique (*1*).

The data in Table 1 are from two experiments in which root tips were treated with H^3-thymidine after removal from 5AU. In experiment A, a treated lot was placed in H^3-thymidine for 1 hour immediately after removal from 5AU. In experiment B, one lot of roots (B-1) was labeled from 1.5 to 3 hours after removal from 5AU, and the other lot (B–2) was labeled from 3 to 4.5 hours. Recovery of mitotic activity appeared to be somewhat more rapid in the experiments listed in Table 1 than in the experiments shown in Fig. 1, probably because the added thymidine overcame the effect of 5AU, but in general the two groups of data are similar in showing that a high frequency of division stages is reached 14 hours after removal from 5AU.

More grains were counted over nuclei labeled 3 to 4.5 hours after treatment with 5AU (B-2) than in nuclei labeled 1.5 to 3 hours afterward (B-1). This finding indicates progressive recovery of DNA synthetic rate following release from inhibiting effects of the pyrimidine analog. The result is not unexpected since 5AU interferes with thymine synthesis and folic acid metabolism in *Allium cepa* (*2*). The H^3 incorporated into interphase S-stage nuclei, as shown in roots fixed immediately after labeling, was observed later (Table 1, 14-hour fixation) in both dividing and interphase nuclei (*3*).

Table 1. Results from labeling *Vicia faba* root tip cells with tritiated thymidine (H^3-thy) after a 24-hour treatment with 5-aminouracil (5AU).

Time (hr)			Fixed immediately after H^3-thymidine labeling			Fixed 14 hours after 5AU treatment		
5AU to H^3-thy	In H^3-thy	Stage	Nuclei (%)	Nuclei labeled (%)	Grains per nucleus (No.)	Nuclei (%)	Nuclei labeled (%)	Grains per nucleus (No.)
			Experiment A					
0	1.0	Interphase	99.8	13.8	3.6	37.5	10.3	7.3
		Division	0.2	0.0	0.0	62.5	28.5	5.3
			Experiment B-1					
1.5	1.5	Interphase	96.2	36.6	5.6	48.8	36.4	7.1
		Division	3.8	0.0	0.0	51.2	57.6	7.0
			Experiment B-2					
3.0	1.5	Interphase	92.5	51.0	14.2	44.1	31.8	15.9
		Division	7.5	0.0	0.0	55.9	46.5	12.2

HAROLD H. SMITH
CATHARINE P. FUSSELL
BERTHA H. KUGELMAN
Department of Biology, Brookhaven National Laboratory, Upton, New York

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Reaction of Lymphocytes with Purified Protein Derivative Conjugated with Fluorescein

Abstract. *Buffy coats from the peripheral bloods of persons known to give a positive reaction to purified protein derivative and of those known to be negative were treated with purified protein derivative conjugated with fluorescein isothiocyanate. Lymphocytes from positive reactors took up the labeled antigen in significantly greater numbers than did those from negative reactors. Leukocytes from newborns showed far less affinity for the antigen than leukocytes from adult negative reactors.*

Passive transfer of tuberculin skin hypersensitivity may be accomplished by transfusing leukocytes derived from spleen, peritoneal exudate, peripheral blood, and lymph nodes from tuberculin reactive animals to nonreactive animals (*1*). Transfusion of plasma was found not to be effective in conferring hypersensitivity upon recipients. Moreover, leukocyte extracts, as well as the intact cells, are capable of transferring hypersensitivity (*2*).

Lymphocytes from guinea pigs sensitized to tuberculin take up I^{131}-labeled purified protein derivative (PPD) to a somewhat greater extent than do lymphocytes from nonsensitized animals (*3*), but it could not be shown that cells from sensitized animals are concentrated, after passive transfer at the site of PPD injection (*4*). The uptake in vitro of PPD labeled with I^{125} by some of the cells teased from thymus, spleen, and lymph nodes of tuberculin-