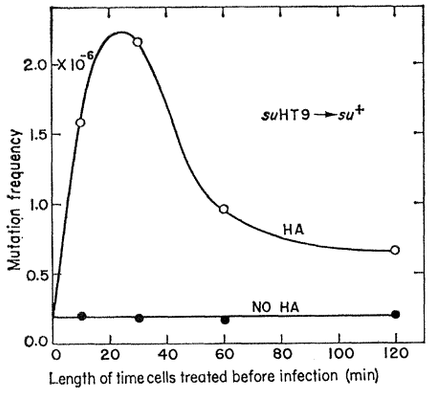

Fig. 1. Mutation frequency of *su*HT9 ⟶ *su*⁺ relative to the number of input phage as a function of the length of time the host cells were treated with hydroxylamine (HA) before infection. *Escherichia coli* C was grown to 1×10^8/ml in tryptone broth (13 g of Bacto tryptone per liter of H_2O), *p*H 6.4. To 20 ml of the bacterial suspension was added 0.5 ml of 1*M* HA at *p*H 6.0. This was shaken at 37°C. At the indicated times, 5.0 ml was removed and centrifuged, the pellet then being resuspended in 5.0 ml tryptone broth, centrifuged again and resuspended to give a cell concentration between 5 and 10×10^8/ml in 1.0 ml tryptone broth-salt (13 g of Bacto tryptone plus 7 g of NaCl per liter of H_2O) containing $1 \times 10^{-2}M$ $CaCl_2$. To 0.2 ml of this bacterial suspension was added 0.05 ml of phage at 1×10^9/ml. Ten minutes at 37°C was allowed for adsorption, and then the entire phage and cell mixture was plated with indicator bacteria for phage assay. A control culture of cells was handled in the same way but with no HA added. Before treatment with HA, approximately 100 percent of the bacteria observable in the microscope could form colonies, but after 120 minutes of treatment, approximately 50 percent could not.

cases the host normally permits growth of the parent phage types. Both mutations were induced.

A similar effect could be obtained in an inorganic buffer. Cells grown in broth were treated with hydroxylamine in 0.1*M* sodium phosphate buffer, *p*H 6.8, containing $10^{-3}M$ $MgSO_4$. The one mutation studied, *su*HT9⟶*su*⁺, was induced. At 10 minutes the effect was about half as great as the value shown in Fig. 1 for broth. But no mutagenic effect was observed at 30 and 60 minutes. The reason could well be the extremely rapid inactivation of cells treated in the buffer; the survival decreased roughly exponentially, reaching 10^{-2} at 60 minutes.

When cells were treated with hydroxylamine, centrifuged, and then aerated in tryptone broth at 37°C, the mutagenic effect of the hydroxylamine was reduced, but even after 80 minutes of aeration there was still a threefold increase in the mutation *su*HT9⟶*su*⁺ compared with control cells not treated with hydroxylamine.

Because hydroxylamine is such a highly specific mutagen in vitro, it might at first seem surprising that it should be so unspecific in vivo. But the mutagenic effects in vivo are actually not unreasonable considering the widespread effects that HA and its derivatives have on *E. coli*. At $10^{-3}M$ concentrations, hydroxylamine is known to seriously affect many distinct cellular processes, immediately stopping DNA, RNA, and protein synthesis (*6*); and inhibitory effects have been observed even at $10^{-4}M$ (*7*). It should not be surprising if such damages were to impair the fidelity of the replication process and thus increase the frequency of many types of mutations.

It is also conceivable that hydroxylamine can react with cytosine to produce an analogue for both cytosine and thymine that is mutagenic. Furthermore, mutagens might be produced by the reactions of hydroxylamine with other substances besides cytosine. For example, a large class of hydroxamates could be formed (*8*), particularly with the aid of intracellular enzymes; and some of the hydroxamates would be expected to react with bases other than cytosine (*9*). Perhaps in that way each base could be chemically modified. But actually, the formation of just one base analogue could be enough to explain the induction of all the mutations observed (*5*). In any case, it is clear that for the in vivo mutagenic effects there are many conceivable explanations that require further study.

The S13 experiments suggest possible limitations of hydroxylamine as a specific cellular mutagen. Hydroxylamine appears to be highly specific for just cytosine in vitro, but cellular mutations are usually induced in vivo. It is possible to mutate some cells by treatment in vitro of transforming DNA or transducing viruses, in which cases we would expect only cytosine to be affected. But if cellular mutations were produced by hydroxylamine treatment of the cells directly, then the in vivo results for phage S13 should alert us to the possibility of a mutagenic effect on any one of the four bases. Other mutagens might also behave quite differently in vivo than they do in vitro.

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Thin-Layer Chromatography of Plant Pigments on Mannitol or Sucrose

Abstract. *Fat-soluble tobacco leaf pigments were separated by chromatography on thin layers of mannitol and sucrose. Resolution of pigments, simplicity, and rapidity of preparation and development offer advantages over previous methods.*

Successful separation of plant pigments has long been obtained by column chromatography on adsorbents such as powdered sugar or powdered cellulose (*1*). Thin-layer chromatography on sucrose (powdered confectioners' sugar containing 3 percent cornstarch) was recently described (*2*). A method with mannitol as the adsorbent has been developed in this laboratory.

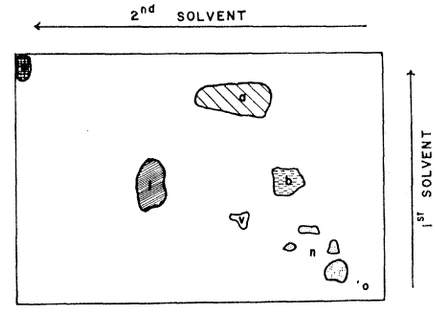

Fig. 1. Diagrammatic figure showing the two-dimensional separation of chloroplast pigments from a young tobacco leaf obtained on a mannitol thin-layer plate. *1st solvent*, 2 percent (by volume) methanol in petroleum ether (b.p. 30° to 60°C); *2nd solvent*, 5 percent (by volume) acetone in petroleum naphtha (b.p. 60° to 70°C); *a*, chlorophyll *a*; *b*, chlorophyll *b*; *c*, carotenes; *l*, lutein; *v*, violaxanthin; *n*, other xanthophyll pigments; *o*, origin.

Suspensions for coating the plates are prepared by blending 65 g of mannitol in 100 ml of acetone for 1 minute in a blender. One milliliter of aqueous cornstarch (5 g in 10 ml) is added to the slurry, which is blended for an additional minute. The suspension is then transferred to a capped bottle in which it may be stored for several days. The inclusion of cornstarch in the mannitol suspensions gives better adhesion and does not appear to influence the separation of the pigments.

Application and spreading of the layer must be carried out very quickly because of the rapid evaporation of the solvent. The plates are ready for use within 20 to 30 minutes, and no oven drying is necessary. The plates may be stored under normal laboratory conditions or in a desiccator.

Sucrose plates can be prepared similarly with a suspension of powdered confectioners' sugar (75 g) in acetone (100 ml) with 1 ml of aqueous sucrose (50 percent wt/vol) being added during blending.

In a comparsion of Colman and Vishniac's method (powdered sugar) and plates coated with mannitol, the mannitol plates gave better separation with less streaking or tailing.

Acetone–water (80:20 by volume) extracts of tobacco leaves were partitioned against petroleum ether. Water was removed from the petroleum ether extracts with anhydrous Na_2SO_4 and portions of these extracts were applied to the test plates. The following solvent systems were used to develop the plates: (i) 0.5 percent (by volume) *n*-propanol in petroleum ether (b.p. 30° to 60°C); (ii) 2 percent (by volume) methanol in petroleum ether (b.p. 30° to 60°C); (iii) 5 percent (by volume) acetone in petroleum naphtha (b.p. 60° to 70°C). All plates were developed for an approximately equal length of time (30 to 40 minutes).

One-dimensional development gave pigment R_F values as shown in Table 1, whereas two-dimensional development on mannitol plates gave a separation as shown in Fig. 1.

These results show that mannitol gave a better separation of the individual bands and separation of several additional yellow pigments in the xanthophyll range below chlorophyll *b*. The number of yellow pigments in this region varied with the solvent but was always greater on the mannitol plates than on the powdered sugar plates. The rate of travel of the solvent front was always greater on the mannitol plates.

Table 1. R_F values of tobacco leaf pigments on thin-layer plates developed in three solvent systems. PE, petroleum ether (b.p. 30° to 60°C); PN, petroleum naphtha (b.p. 60° to 70°C); PS, powdered sugar; M, mannitol. The numbers in parentheses are distances in millimeters traveled by the solvent front.

Pigment	2% Methanol in PE		0.5% *n*-propanol in PE		5% Acetone in PN	
	PS(110)	M(156)	PS(114)	M(157)	PS(115)	M(152)
β-Carotene	0.98	0.99	0.97	0.99	0.97	0.99
Chlorophyll *a*	.90	.82	.82	.73	.35–.52*	.28
Lutein	.80	.57	.61	.55	.63	.57
Chlorophyll *b*	.64	.50	.44	.40	.26	.17
		Xanthophyll pigments				
	0.30–0.54*	0.46	0.20–0.40*	0.30	0.50–0.60*	0.44
		.40		.13		.37
		.31		.07		.10
		.24		.04		
		.20				
		.15				

* Pigments did not separate but streaked over a range of R_F values.

Mannitol plates can be used to separate the isomerization and breakdown products of the chlorophyll pigments, such as the pheophytins, chlorophyll a^1, and chlorophyll b^1 from the undegraded pigments. When leaf extracts stand for several hours at room temperature, the gray pheophytin band appears just behind that of β-carotene, and chlorophyll *a* and *b* separate into two distinct bands.

The method has been used successfully to separate the products of chlorophyll degradation produced by γ-irradiation of chloroplasts (*3*).

The thin-layer plates offer advantages to column methods because they are simple to prepare and use. Mannitol plates require only about 20 minutes for 12-cm development with a mixture of propanol and petroleum ether; the high resolution obtained facilitates removal of the pigment bands, without cross contamination, for quantitative work.

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18 February 1965

Hemoglobin Heterogeneity: Embryonic Hemoglobin in the Duckling and Its Disappearance in the Adult

Abstract. *The 7-day white Peking duck embryo contains two hemoglobins (Hb's III and IV) which are electrophoretically distinct from the two adult hemoglobins (Hb's I and II). Hemoglobin IV had disappeared in the 14-day embryo, at which time Hb's I and II are evident. The synthesis of Hb III is not restricted to embryonic life but continues in the duckling and ceases before the 10th week of development after hatching.*

In 1955 Cabannes and Serain demonstrated the presence of two hemoglobins in the duck (*1*). Other investigators have confirmed this observation using alkali denaturation (*2*), agar electrophoresis (*3*), and ion-exchange chromatography (*4*). We now report a third hemoglobin in the duck. This hemoglobin originates in the embryo; its synthesis continues after hatching but ceases in the adult.

Ducklings were obtained within 24 hours after hatching; they were bled by cardiac puncture. Adult ducks were bled from the wing vein, and embryo blood was obtained by severing the omphalomesenteric vessels. The eggs containing 7-day-old embryos were opened close to the air space (blunt end of egg), and the yolk was allowed to drain. Adhering material was removed with scissors and forceps. We endeavored to keep the embryo and as much of its circulatory system as possible fixed to the inner shell which served as a retainer when the blood