The inducer is not directly responsible for the inhibitory effect of induction on intragenic recombination shown in Fig. 2, since the introduction of regulator constitutive mutations into the merodiploid has the same inhibitory effect (*1*). Moreover, the repressor molecule cannot be directly involved since the strongly polar mutants have wild-type regulator genes, and the removal of inducer does not increase recombination in these strains (Fig. 1). The control of the *lac* operon appears to operate most directly at the level of transcription (*4*); therefore, it seems very likely that the synthesis of lac mRNA is involved in the inhibition of intragenic recombination.

Translation of nascent mRNA molecules into polypeptides may begin before the synthesis of the message is completed (*5*). The nascent mRNA with its associated ribosomes is conceivably somehow responsible for the inhibitory effect of induction on recombination. If this were true, intragenic recombination in strongly polar mutants might be unaffected by induction, even if *lac* mRNA synthesis were induced normally, since the polar mutation could lead to release of ribosomes from the nascent message and rapid degradation of the message beyond the nonsense codon. But the *Z* gene is nearly as long as an *E. coli* cell (*6*).

It seems unlikely that the structure of the gene or its accessibility for participation in recombination with its homolog would be much affected by its nascent mRNA-ribosome complexes. Therefore, I favor the view that transcription alone inhibits intragenic recombination in merodiploids. It follows from this interpretation and the results illustrated by Fig. 1 that strongly polar mutations prevent normal transcription. This view is also favored by the work of Imamoto and Yanofsky on the kinetics of transcription of the tryptophan operon in polar mutants (*7*).

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2 April 1968

Crystals from Cocoons of Malacosoma neustria testacea

Abstract. *The crystalline material covering the cocoon of* Malacosoma neustria testacea (*Lasiocampidae, Lepidoptera*) *was analyzed physically and chemically. The mean component was identified as calcium oxalate monohydrate, and the crystals were found to be whewellite in form.*

It has long been known that a crystalline powder covers the cocoons of some Lepidoptera (*1*), but the detailed nature of this material has not been worked out. We have analyzed the crystalline material of the tent caterpillar *Malacosoma neustria testacea* and found it to be calcium oxalate monohydrate.

About 400 cocoons were collected on the campus of Nagoya University and were repeatedly washed with 60 percent aqueous methanol. The suspension was centrifuged, and the yellow material packed in the bottom of the tube was dried in a desiccator under reduced pressure. The powder is composed mainly of crystalline material (Fig. 1).

Since preliminary analyses of the material revealed that the main component was calcium oxalate, calcium was estimated gravimetrically after the material was dissolved in dilute HCl. The calcium was precipitated with ammonium oxalate, and the oxalate was calcined in an electric furnace. Its content amounted to 33.7 percent by weight when calculated as CaO, or 87.7 percent when calculated as calcium oxalate monohydrate (*2*).

The content of oxalic acid was determined by titration with potassium permanganate. The material was dissolved in dilute HCl and passed through a Dowex-50 column. The fraction not adsorbed was neutralized, and the acid was precipitated with calcium chloride. The calcium salt, after being collected by centrifugation and washed repeatedly, was dissolved in dilute H_2SO_4 and titrated with permanganate. The material that could be titrated, with permanganate amounted to 80.6 percent when calculated as calcium oxalate monohydrate. The material also contained iron which was estimated colorimetrically with α,α′-dipyridyl, and the content was 0.35 percent by weight (*2*).

Identification of the material as calcium oxalate monohydrate was further confirmed by infrared spectrophotometry: the sample and the reagent of calcium oxalate monohydrate gave the identical pattern (*3*). The x-ray powder diffraction pattern for this material agrees well with that of whewellite (*4*), which is given in the ASTM card (Table 1) (*5*).

Table 1. X-ray powder diffraction data for cocoon crystals and $CaC_2O_4 \cdot H_2O$ (whewellite) (*5*); *d* (Å), spacing in angstroms; *I*, intensity.

Cocoon crystals		$CaC_2O_4 \cdot H_2O$ (whewellite)	
d (Å)	I/I_1	d (Å)	I/I_1
5.97	100	5.93	100
5.83	40	5.79	30
4.79	4	4.77	2
		4.64	1
4.55	10	4.52	4
3.79	20	3.78	6
3.66	100	3.65	70
3.35	10	3.41	2
		3.12	2
3.12	4	3.11	2
3.00	30	3.01	10
2.96	70	2.966	45
2.92	30	2.915	10
2.90	20	2.897	8
2.84	30	2.840	10
2.52	10	2.523	4
2.50	50	2.494	18
2.44	10	2.447	4
2.42	10	2.417	6
2.39	20	2.384	4
2.36	60	2.355	30
2.35	50	2.347	12
		2.320	1
2.30	4	2.301	2
2.26	40	2.263	8
		2.254	6
2.21	10	2.210	6
2.13	6	2.130	2
2.09	10	2.089	2
2.08	40	2.075	14
2.00	10	1.995	2
1.98	20	1.978	10
		1.957	2
1.95	30	1.950	10
1.93	20	1.933	8
		1.923	2
1.89	20	1.890	6
1.86	10	1.859	4

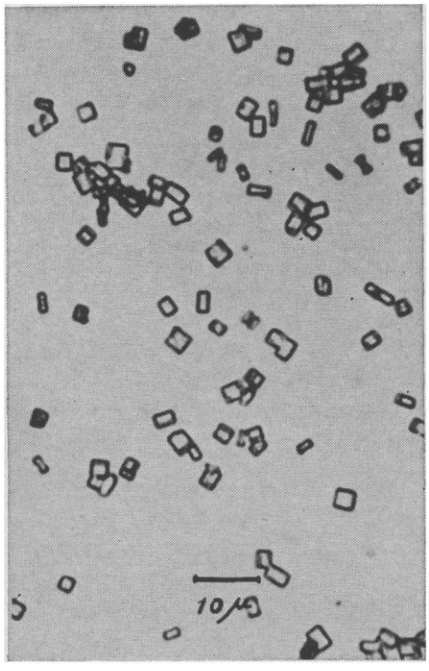

Fig. 1. Crystals from the cocoons of *Malacosoma neustria testacea.*

The crystals originate from the contents of Malpighian tubes and have erroneously been considered to be uric acid by other workers (*6*). The yellow pigment is assumed to be impregnated after the crystallization of calcium oxalate within the lumen of the tube. The oxalate amounts to more than 5 mg per individual. The indications are that this compound is concerned with the regulation and excretion of calcium by this insect.

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Stomatal Opening: Role of Potassium Uptake by Guard Cells

Abstract. *Stomata in isolated epidermal strips open in response to light plus air free of carbon dioxide when the strips are floated on potassium chloride solutions of low concentrations. This opening depends on the stimulation of active accumulation of potassium in quantities sufficient to account for the observed changes in solute potential of the guard cells.*

This study of stomatal opening in isolated epidermal strips provides new evidence that opening is due to active accumulation of potassium by guard cells. Essential to the relevance of this finding was the prior demonstration that stomata in such strips are living and respond normally to natural stimuli such as light and changing concentration of CO_2; normal opening had not been demonstrated during extensive earlier work with epidermal strips (*1*, *2*).

Abaxial epidermal strips (0.3 by 0.4 cm) of *Vicia faba* were used, being largely free of entire mesophyll cells although some mesophyll cell fragments and chloroplasts usually adhered to the strips. The proportion of epidermal cells remaining intact, judged by accumulation of neutral red and appearance of the protoplasm, was kept below 20 percent and did not appear to influence stomatal movement. Almost all guard cells were living as judged by accumulation of neutral red and presence of protoplasmic streaming.

Stomata in strips floated on certain solutions responded to light plus CO_2-free air in the same manner as did unisolated stomata (Table 1); response applied to changes in aperture, guard-cell solute potential, and guard-cell starch content (determined by scoring after iodine staining). The treatment with light plus CO_2-free air was used because it is generally thought that the opening by light of stomata depends partly on the reduction in concentration of intercellular CO_2, and thus that the light response of stomata in isolated epidermal strips is not fully expressed unless the CO_2 concentration of the environment is reduced similarly. For convenience, CO_2-free air (<0.0001 percent CO_2 by volume) was used; in fact other experiments (*3*) showed that, although stomata in strips responded independently to light and to CO_2-free air, only with both factors together did stomatal apertures equal those of unisolated stomata under comparable conditions.

These results demonstrate normal function of stomata in isolated epidermal strips and justify further studies with this system. It was found that the only component of the buffer solution (Table 1) that was essential for the opening of isolated stomata in light plus CO_2-free air was KCl; without KCl the opening response is slight (Fig. 1). Replacement of potassium with calcium inhibited opening completely, whereas substitution of sulphate for chloride reduced opening by only 30 percent. The important component thus appears to be potassium.

For investigation of the importance of the concentration of potassium (Fig. 1), the opening under light plus CO_2-free air was saturated by approximately 10 m*M* KCl; opening occurred in the dark with normal air also, but required 100 m*M* KCl for saturation. Stomatal opening was markedly reduced with KCl at 200 or 400 m*M*.

The essentiality of potassium and the form of the response to changes in its concentration suggested that stimulated uptake of potassium was implicated in stomatal opening. This uptake was

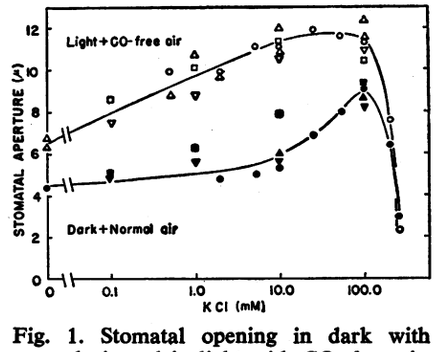

Fig. 1. Stomatal opening in dark with normal air and in light with CO_2-free air, and the response to concentration of KCl. Light intensity, 2150 mphot; temperature, 31° to 34°C; stomata measured after 180 minutes in light or dark. All solutions contained 2.5 m*M* tris-maleate buffer and 0.5 to 0.7 m*M* Ca^{2+}. Results of various experiments, each designated by a different symbol and involving five to 16 replications; all results were corrected to give identical mean apertures in dark plus normal air and zero KCl (that is, 4.4 μ). Before correction, mean aperture under this condition ranged from 2.9 to 5.7 μ for the various experiments; correction involved the addition of the difference between 4.4 μ and this value for each experiment to the mean stomatal aperture at each other concentration of KCl in the experiment. Initial stomatal apertures averaged 1.0 μ less than the final values in the dark with normal air and zero KCl.