Group	No. of perfusions	Treatment*	Cholesterol CPM/g liver (±S.E.)	% inhibition	Fatty acids CPM/mg fatty acids (± S.E.)	% inhibition
1 2 3 4	7 5 4 4	Controls	$   \begin{array}{c}     1801 \pm 230 \\     1031 \pm 103 \\     4403 \pm 417 \\     2311 \pm 218   \end{array} $	$\frac{42\cdot7}{47\cdot5}$	$204 \pm 28$ $146 \pm 22$ $342 \pm 56$ $212 \pm 26$	28·4 

CPM = counts per min.

Levels of probability: for cholesterol group 1–2: p < 0.05; 1–3: p < 0.001; 1–4: p > 0.05; 3–4: p < 0.002; for fatty acids group 1–2: p > 0.05; 1–3: p < 0.01; 1–4: p > 0.8; 3–4: p < 0.05

\* DFLEA was kindly supplied by Maggioni Laboratories, Milano, and Triton W. R. 1339, a polymer of p-iso-octylpolioxyethylenphenol, by Rohm and Haas Co., Philadelphia.

or in the hepatic tissue. These data represent a contribution for an analysis of the mode of action of Triton.

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Institute of Pharmacology, University of Milan (Italy), June 20, 1958.

## Riassunto

L'acido difenililetilacetico antagonizza, nel fegato perfuso di coniglio, l'aumento di incorporazione dell'acetato-1-C<sup>14</sup>, nel colesterolo enegli acidi grassi, indotto dal Triton.

## Temperature-Induced Reversal of Dominance of Variegation in 'Ornamental Kale'

Among the auxotrophic mutants of *Neurospora*, approximately 8% are temperature-sensitive. It is therefore not surprising that temperature-sensitive mutants occur in higher plants. Examples which have been reported include recessive albinism in barley (Collins¹), flower-pigmentation in *Primula sinensis* (Baur²), and thiamineless in *Arabidopsis thaliana* (Langridge³). This report concerns a mutant of *Brassica oleracea* L. which has unusual features resulting from its temperature-sensitivity.

'Ornamental kale' is a variety of Brassica oleracea L. in which the leaves become variegated during the winter in South Australia. Seeds sown in late summer produce wholly green seedlings. In early winter, white tissue appears near the midrib and spreads outwards. In spring, the white parts become green; i.e. the albinism is reversible. In some plants all the leaves may become quite white except for a narrow green margin, while the inflorescences appearing in spring are also devoid of chlorophyll. At the other extreme, the albinism may be confined to the midrib and a narrow strip alongside it in the floral bracts, while the inflorescences in such plants are usually green except for white patches below the bases of the pedicels. Thus there is great variation in the extent of the white tissue. The date when white tissue first appears also varies, e.g. in 1955, from May 10 to August 11. Generally speaking, earliness of appearance is correlated with a large amount of white tissue and vice versa.

A plant showing extensive and early variegation and subsequently shown to be true-breeding for the character, was crossed to broccoli (Yates Green Sprout). Meiosis in the F1 appeared to be normal, nine bivalents being formed. Six F1 plants from each of the reciprocal crosses were sown on March 6, 1955, planted out into the field on April 13 and all showed variegation within three days of June 2. The extent of variegation was quite uniform, there being a narrow margin of white tissue alongside the midribs of the inner leaves of the rosette.

A further 16 plants from the same cross were planted in pots at the same time and divided between four treatments viz.

Long day (18 h) in the glasshouse.

Long day (18 h) and outdoor temperatures.

Natural day  $(10-11^1/2 \text{ h})$  in the glasshouse. Natural day and outdoor temperatures.

The plants in the long day grew more quickly than those in the short day but the length of day made no difference to the date when albinism first appeared. Temperature had a marked effect. All 8 plants outside the glasshouse showed variegation about a week after their sibs in the field (i.e. on June 10). By July 13, those in the glasshouse still showed no sign of variegation. Half those inside the glasshouse were then placed outside and vice versa. The 4 variegated plants from outside, on being placed in the glasshouse, started to become green immediately and were almost normal after 14 days. The 4 green plants from the glasshouse showed the first signs of variegation, a narrow white strip alongside the midribs of the inner leaves, from 14 to 18 days after being placed outside. The 4 left inside the glasshouse and the 4 left outside showed no change.

Variegation seems to be a response to temperature, although what the critical temperatures are, is uncertain. The glasshouse was heated to  $21^{\circ}$ C. The outside temperatures during the 14 days between removal from the glasshouse and the appearance of variegation ranged from a mean daily maximum of  $13\cdot2^{\circ}$ C to a mean daily minimum of  $7\cdot7^{\circ}$ C.

In all, 24 F1 plants (12 in the field, 12 in pots) were exposed to outside temperatures and all showed variegation. All 41 F1 plants from another similar cross were also variegated. It is reasonable to assume that in this season the character was always expressed. In a F2 family growing at the same time there were 73 variegated and 25 non-variegated plants. In the progeny from the backcross to broccoli there were 26 variegated and 22 non-variegated plants. The difference between variegated and non-variegated plants is apparently controlled by a single gene difference, the variegated allele being dominant. Moreover, because the variegated backcross and F1 plants are always of the less variegated type while the extreme types are true-breeding for variegation, there seems to be

<sup>&</sup>lt;sup>1</sup> J. L. Collins, J. Heredity 18, 331 (1927).

<sup>&</sup>lt;sup>2</sup> E. BAUR, Einführung in die experimentelle Vererbungslehre, 11th Ed. (Berlin 1930).

<sup>&</sup>lt;sup>3</sup> J. Langridge, Nature 176, 260 (1955).

a gene dosage effect. However, the phenotypic distinction between the two genotypes is not always clear.

During the winter of 1954 another sample of the same F2 family had been grown, giving 31 variegated and 25 non-variegated plants, while in another backcross family there were 10 variegated and 20 non-variegated plants. Assuming that the character was always expressed by homozygotes but only by a proportion p of heterozygotes, the method of maximum likelihood gives a value of p of 0.63 with 95% fiducial limits of 0.38 and 0.88. If 37%of heterozygotes were non-variegated, then approximately 10 of the 25 non-variegated F2 plants should have been heterozygotes. 5 of these plants were tested by selfing and 8 by backcrossing to proven homozygous non-variegated plants. The resulting families were grown in 1955 and 4 of the 13 plants tested were shown to have been heterozygotes because they segregated the expected proportions of variegated progeny. The only obvious seasonal difference between 1954 and 1955 to which the difference could be attributed was the colder temperature in May, the mean daily maxima and minima being 2°C lower in 1955.

Another sample of this F2 family was sown in November 1956. During the ensuing summer only 5 out of 33 plants showed the variegation and these only to a limited extent. The ratio variegated: non-variegated is approximately 1:3. It seems probable that the variegated plants were homozygotes and that the character was not expressed in the heterozygotes. During summer variegation was apparently recessive. There was a reversal of dominance.

These experiments show that variegation in 'Ornamental kale' is not only temperature-sensitive but, in addition, the frequency with which it is expressed differs in homozygotes and heterozygotes. The interaction of these two features may give rise to the phenomenon of reversal of dominance at different temperatures.

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Department of Zoology, University of Adelaide (Australia), August 29, 1958.

## Résumé

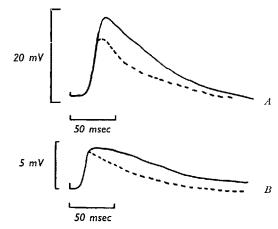
La transmission de l'albinisme panaché de Brassica oleracea L. ne dépend que d'un seul facteur. Chez les hétérozygotes ce caractère apparaît à basse température (7°C), mais pas à haute température (21°C). Chez les homozygotes le caractère se manifeste probablement toujours, tout en restant peu prononcé à des températures plus élevées. Ainsi il y a une inversion du caractère dominant avec changement de température.

## The Effects of γ-Aminobutyric Acid and Picrotoxin on the Junctional Potential and the Contraction of Crayfish Muscle<sup>1</sup>

Stimulation of the inhibitory neuron of the opener muscle of the crayfish claw normally reduces the contraction. But the inhibition resulting from this stimulation is

<sup>1</sup> The study was supported by Grants B-1089 and B-31 from the National Institute of Neurological Diseases and Blindness, United States Public Health Service. Some of this work was done at the Department of Zoology, Cornell University. We are grateful to Prof. S. C. Wang, Columbia University, for generous assistance.

greatly reduced in the presence of picrotoxin<sup>2</sup>. It is also known that  $\gamma$ -aminobutyric acid (GAB) inhibits the contraction of crayfish muscle<sup>3</sup>. It therefore seems possible that GAB mimics the action of the inhibitory transmitter on the muscle. This idea would be supported if the action of GAB, like that of the inhibitory transmitter, were blocked by picrotoxin (the blocking of GAB's action by picrotoxin has been demonstrated on the Crustacean heart<sup>4</sup>).



A and B Junctional potentials recorded with an intracellular electrode before (——) and after (+-+) the perfusion of the muscle with  $2\cdot4\times10^{-4}\,M$  GAB

Our results  $^5$  show that when GAB was introduced into the perfusion fluid, the response by the muscle to stimulation of the motor neuron at 60/s was slightly reduced by concentrations of GAB as low as  $3\cdot7\times10^{-6}\,M$  (0·38 µg/ml); invariably, contractions were completely eliminated by  $10^{-3}\,M$  GAB. The GAB effect was readily reversed by washing the claw free of the amino acid or by perfusing with a solution containing  $10^{-4}\,M$  picrotoxin along with the GAB. From this result it is clear that picrotoxin does block the inhibitory action of GAB on crayfish muscle.

This evidence by itself does not prove that GAB is the inhibitory transmitter, because contractions were also blocked completely by other amino acids,  $10^{-2}~M~\beta$ -alanine for example. And the action of  $\beta$ -alanine was also blocked by picrotoxin.

We have studied the effects of GAB and of picrotoxin on the membrane of the closer muscle by the use of intracellular electrodes. After  $2\cdot 4\times 10^{-4}\,M$  GAB was perfused around the muscle, there was little change in the resting potential of the muscle fiber. But when the 'fast' motor neuron was stimulated while GAB was present there was usually a distinct decrease in the height of the junctional potential (Figure A). And GAB also produced a pronounced increase in the rate of decay of the junctional potential, which suggests that GAB reduces the resistance

<sup>&</sup>lt;sup>2</sup> W. G. Van der Kloot, J. Robbins, and I. Cooke, Science 127, 521 (1958). – J. Robbins and W. G. Van der Kloot, J. Physiol., in press.

in press.

3 J. A. Brockman, Jr. and S. L. Burson, Jr., Proc. Soc. exp. Biol. N. Y. 94, 450 (1957).

<sup>&</sup>lt;sup>4</sup> E. Florey, Naturwissenschaften 44, 424 (1957).

<sup>&</sup>lt;sup>5</sup> The crayfish studied were Cambarus clarkii, C. virilus, and Orconectes immunis.

<sup>&</sup>lt;sup>6</sup> A. Bazemore, K. A. C. Elliott, and E. Florey, Nature 178, 1052 (1956), made the important discovery that GAB and  $\beta$ -alanine block impulse generation in the Crustacean stretch receptor. Apparently GAB mimics the action of the inhibitory transmitter on the receptor, C. Edwards and S. W. Kuffler, Fed. Proc. 16, 34 (1957).