Since the mice and weasels are stimulated to activity by artificial dusk and inhibited by artificial dawn, natural twilights probably are the chief factor influencing onset and cessation of their activity. While artificial dawn stimulates chipmunk activity, artificial dusk merely slows it down. This suggests that natural dawn stimulates these animals to activity but that factors other than dusk inhibit them late in the day. The fact that dim light and darkness merely slow down chipmunk activity in the laboratory is consonant with adaptations of these rodents for living in and fleeing through dark burrows. A detailed report of these studies will appear elsewhere[8,9].

Zusammenfassung. Die Aktivität des Backenhörnchens (*Tamias striatus*) und des Wiesels (*Mustela rixosa*) wurde im Laufrad bei künstlicher Dämmerung und abruptem Tag-Nacht-Wechsel untersucht. Sowohl die Geschwindigkeit als auch die gesamte Laufzeit war bei diesen Tieren genau wie bei nachtaktiven Mäusen (Gattung *Peromyscus*) von der Beleuchtungsstärke abhängig. Die Bedeutung dieser Befunde für das zirkadische Gesetz und die Evolution von Tages- und Nachtaktivität wird diskutiert.

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Hormonal Control of Colour Changes in Orange Peel

The recent years have broadened our concepts about substances which seem to play a hormonal role in plants and the list of processes which appear to be regulated by plant hormones has also grown considerably. Recent reports indicate that the colour changes associated with the ripening of fruits are also hormone controlled[1-4]. In the present communication we shall discuss some experiments conducted with Shamouti oranges (*Citrus sinensis* L.) harvested while still green. Growth regulators were applied to the fruits by dipping them into solutions consisting of 50% ethanol + 0.02% tween 80 and the desired growth substance. In a preliminary experiment we made sure that neither the ethanolic solution nor the detergent had apparent effects on the colour changes in orange peel. The changes in colour were detected by a Hunter Color Difference Meter (Gardner Laboratories Inc.) and the *a*/*b* ratio (*a*, a measure of redness; *b*, a measure of yellowness) served for the quantitative estimation of colour development[5].

Figure 1 shows the effects of several growth regulators, at concentrations of 8 and 80 ppm, on colour development of green harvested oranges, as compared with control. The auxin α-naphthalene-acetic acid (NAA) had almost no influence. Benzyl adenine (BA), which acts as a cytokinin, delayed the colour changes and this is no surprise since cytokinins act similarly in preserving the chlorophyll of detached leaves and delaying their senescence[6,7]. Gibberellic acid (GA_3) has already been known for several years to inhibit colour development in citrus fruits[4,8], and recently it became clear that it has the same effect also on tomatoes[1,3] and bananas[2]. Figure 1 shows the inhibitory effect of 80 ppm and 8 ppm GA_3. The Table shows that even much lower concentrations, down to 0.1 ppm GA_3, still have considerable inhibitory effect.

The effect of GA_3 might be easily obtained also when treatments are given to fruit attached to the tree[4] and in our experiments attached fruit remained green for more than 6 months after the time of colour break. Detached fruit treated with GA_3 attained satisfactory colour only after at least 2 months, whereas control fruit completed the process within 3 weeks. The inhibitory effect of GA_3 could be overcome by ethylene; GA_3-treated fruit held successively at 20 ppm ethylene needed 17 days to attain an almost satisfactory colour. Ethylene has been known for a long time to enhance colour changes and other ripening processes in fruits, and it appears that ethylene and GA_3 act antagonistically to each other in this case.

GA_3 is effective also when applied to a limited section of the peel. The fruits seen in Figure 2 have been harvested while still green and painted with GA_3 solution inside the marked circle. During a month of storage the fruit developed its typical orange colour, except for the treated zone, where the usual effect of GA_3 was obtained. Such local effects were obtained also by applying kinetin[9] and 2,4-dichlorophenoxyacetic acid[7] to restricted areas of detached leaves, thereby delaying the senescence in these areas. The results described in Figure 2 indicate that the process of colour development in peel may be controlled separately in each section of the peel or even in each individual cell. Since the effect of GA_3 is a local one it seems reasonable to relate the amounts of applied GA_3 to the surface unit of the peel. The oranges we treated had a surface of about 150 cm^2 and they adsorbed about 0.9 ml from the applied solution. Since 0.1 ppm GA_3 solution still had an effect (Table) it might be calculated that each cm^2 received not more than 6×10^{-4} μg GA_3 and actually only part of this amount penetrated the peel.

These very low amounts are not higher than the levels of endogenous gibberellins in plant tissues[10] and the sensitivity of the orange peel tissues to GA_3 might be

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Effect of increasing concentrations of GA_3 on colour development of detached oranges

GA_3 concentration	0 (control)	0.1 ppm	1.0 ppm	10.0 ppm
Reflectance[a]	-0.165 ± 0.040	-0.255 ± 0.031	-0.388 ± 0.034	-0.467 ± 0.031

Measurement taken 2 weeks after picking of green fruit. Each value represents an average of 15 fruits, accompanied by its standard error.
[a] *a*/*b* reflectance units measured by 'Hunter' Color Difference Meter.

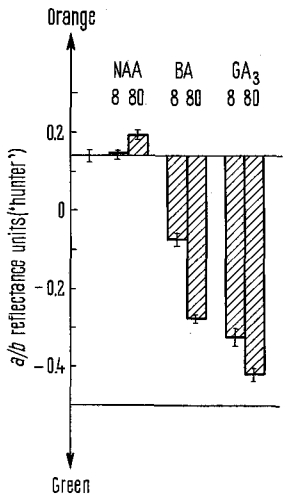

Fig. 1. Effect of various concentrations (8 and 80 ppm) of α-naphthaleneacetic acid (NAA), benzyl adenine (BA) and gibberellic acid (GA_3) on colour development of green-harvested oranges. The lower line indicates the colour of the fruit at the beginning of the experiment, on the date of harvest; the upper line indicates the colour of control fruit after 2 weeks of storage. Each column represents an average of 20 fruits ± the standard error. *a*, measure of redness; *b*, measure of yellowness.

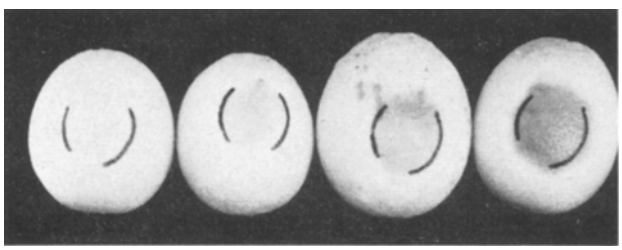

Fig. 2. Effect of various concentrations of GA_3 applied to limited sections of fruit peel, on colour development of green-harvested oranges, after 1 month of storage. From left to right: control (50% ethanol + 0.02% tween 80), 8 ppm, 25 ppm and 80 ppm GA_3.

compared with that of the most sensitive bioassay materials for gibberellins[11,12].

The hypothesis might be advanced that endogenous gibberellins control the colour changes in the ripening fruit, perhaps through a balance with ethylene. Gibberellins and cytokinins might be the endogenous factors which inhibit ripening of fruit before harvest while it is still connected with the tree[13,14].

The small amounts of gibberellins and cytokinins which arrive from the root system with the stream of transpiration[15-17] might suffice to prevent colour changing of the fruit. Several phenomena associated with citrus fruit colouration might be easily explained according to this hypothesis. The requirement of low soil temperatures for colour development[18] might be connected with an inhibition of root growth due to the low temperature and hence, a lack of root-hormones supply for the fruit. The regreening of Valencia orange fruit in the spring[19,20] might also be adequately explained.

We have already pointed out that the hormonal control of peel colouration might be exerted in each individual cell. We might even go a step further. The carotenoid pigments of the ripe orange are located in chromoplasts which appear to derive from chloroplasts which have degenerated and shifted their synthetic activity toward the production of carotenoids instead of chlorophyll[21,22]. Gibberellins have recently been shown to be associated with the chloroplast fraction of leaf homogenates[23]. It seems attractive to propose the idea that the chloro-chromoplast, a subcellular unit, is hormone controlled, by a balance between gibberellins (and/or cytokinins) which tend to preserve the chloroplast as a green photosynthetic unit, and ethylene which leads to the development of chromoplasts, rich with carotenoid pigments typical of ripe fruits and senescent leaves[24,25].

Résumé. Des quantités très faibles d'acide gibbérellique (6×10^{-4} µg/cm²) produisent pendant plusieurs semaines une inhibition de la coloration naturelle de la peau d'oranges vertes détachées. Des résultats semblables ont été obtenus avec une cytokinine. L'inhibition produite par la gibbérelline peut être levée par l'éthylène. On propose que la synthèse des pigments dans le chromo-chloroplaste soit réglée par un contrôle hormonal.

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