skeletal pieces in the post-axial outgrowth were steadily greater than those of the 2nd digit of a merely ap-reversed hand. In several embryos a well recognizable 3rd digit, or the proximal part of the 3rd and 4th digit, developed post-axially and pre-axially. The feather pattern of the surfaces of the twinned hand was dorso-ventrally reversed; the feathers of the post-axial (originally pre-axial) border of the wing apex appeared symmetrical to those of the pre-axial border.

In the Figure 3 examples of twinning of the ap-reoriented apex of the right wing bud in embryos of the stages 24–25 are reported. In (a) the apical ridge was preserved, in (b) and (c) the apical ridge was removed 5 and 6 h respectively after apex reversal. The wings are seen from the dorsal aspect.

These findings indicate that twinning of the hand takes place under the conditions of the present experiment, although variously severe deficiencies of the terminal skeletal and cutaneous structures occur in the ap-reoriented (present experiment) as in the normally oriented apex of the wing bud deprived of its ectodermal thickening[7,8]. It can be concluded that twinning of the ap-reoriented prospective autopode does not depend on the presence and on the inductor activity of the apical ridge of the ectoderm. Apparently, it is the consequence of direct influences exerted by the proximal territories of the limb bud on the growth and the organogenesis of the apical mesoderm.

Résumé. L'excision de la crête apicale 5–10 h après l'inversion de l'axe antéro-postérieur de la partie distale du bourgeon de l'aile n'empêche pas le développement de duplications terminales. Ces duplications seraient dues au jeu d'influences organogénétiques des régions proximales de l'ébauche s'exerçant directement sur le mésoderme apical.

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A Metabolic Pathway of Gramine in Barley

In the last 20 years a great deal has been learned about the biogenesis and metabolism of alkaloids. Unfortunately the function of alkaloids in plants still remains a mystery.

Some interesting approaches to this problem deal with the translocation, distribution, degradation and the relationship between alkaloid content and age of the plant. An excellent account on these aspects of alkaloid biochemistry is that of MOTHES[1].

We wish to report here our recent findings on gramine, which may perhaps throw more light on the metabolism of this substance in barley.

The alkaloid gramine, which is formed in germinating barley from tryptophan[2–5], appears in barley shoots on the 3rd day after germination and remains there in detectable quantities until the 50th day[6]. We have experienced this phenomenon while experimenting with a native strain of Lebanese barley (designated as Baladi 25, Telamara) attributed to *Hordeum distichon* L. When we administered gramine (labelled in the carbon attached to the ring) to the above barley (60 days old), 0.84% of the alkaloid was converted into tryptophan[7]. In the course of this biosynthesis study we were impressed by the fact that no labelled gramine was recovered from the plant; we therefore undertook to examine the possibility of the biological degradation of gramine's methylene carbon to carbon dioxide.

In a representative experiment, five 60-day-old shoots, cut very close to their grain and washed thoroughly, were allowed to stand in a cup within a bottle (covered with a black cloth) containing 1.98 mg of labelled gramine[8] ($10.5 \cdot 10^6$ dpm/mg) dissolved in 2 ml of $5 \cdot 10^{-3}$ *N*-acetic acid[9]. 5 ml of distilled water were added daily to the cup and, when nearly all was absorbed, another 5 ml were added in order to attain the greatest possible absorption of the radioactive material. Carbondioxide-free air was allowed to carry the carbon dioxide expired by the plants into 2 barium hydroxide traps. The precipitated barium carbonate was collected every 6 h for radioactivity counting[10]. Since the carbonate was found to be highly radioactive, samples were collected for 8 days. The plants were then removed from the bottle, washed thoroughly, dried (at 50 °C for 24 h) and subsequently cut into small pieces to produce 2.4 g of herb.

Very little radioactive gramine[11] ($1.0 \cdot 10^4$ dpm) was recovered when a sample (1.2 g) of the herb was analysed by a method similar to that of GOWER and LEETE[5]. When the protein fraction of another plant sample (1.2 g) was examined, according to our previous method[7], it was found that 0.4% of the gramine was incorporated into tryptophan. An aliquot from the total $BaCO_3$ (collected during 8 days) was converted to CO_2, which was trapped

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[8] Gramine, labelled in the carbon attached to the ring, was synthesized from indole, dimethylamine and C^{14} paraformaldehyde according to the procedure of H. KUHN and O. STEIN, Ber. dt. chem. Ges. *70*, 567 (1937). The C^{14} gramine was purified by 2 sublimations, and after 1 crystallization from *n*-hexane was shown to be pure by mixed melting point with authentic gramine, UV-spectroscopy and thin-layer chromatography in 2 solvents. Subsequent radioautography of the chromatograms revealed only one radioactive spot.
[9] All radioactivity measurements unless otherwise specified were performed in a liquid-scintillation counter with an efficiency of 70% and a background count of 12 counts/min.
[10] The barium carbonate samples were plated at infinite thickness and were counted in a thin-window gas-flow counter (Hewlett-Packard Model 5202 L).
[11] The identity of the recovered gramine was proved by chromatography (TLC) in 2 solvents.

by a methanolic solution of hyamine hydroxide according to a procedure similar to that of PASSMANN et al.[12], and subsequently counted. It was found that 10% of the metabolized gramine was incorporated into expired CO_2.

When the above experiment was repeated with the grain remains of five 62-day-old plants, no radioactive CO_2 was detected. Likewise no radioactivity was observed in the expired CO_2 when radioactive skatole[13] (3-methylindole) was provided to barley under identical conditions to those described above.

The above experiments suggest that the alkaloid gramine is metabolized by excised barley shoots (grown in the dark), and that one of its metabolic pathways[14] is the oxidation of its methylenic side chain to carbon dioxide. We are currently attempting to isolate intermediates of this oxidative pathway[15].

Résumé. Nous avons observé que quand la gramine marqué au carbone attaché au noyau hétérocyclique a été administrée à des pousses d'orge agées de 60 jours (dans l'obscurité), 10% de la radioactivité a passé au CO_2 expiré et 0.4% au tryptophane.

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School of Pharmacy, American University of Beirut, Beirut (Lebanon), 7th March 1967.

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[14] Very little incorporation of gramine into the lipid fraction of the plant was observed.

[15] This investigation was supported by a grant from the University Medical Research Fund for which one of us (G.A.D.) is grateful. We are grateful to Dr. C. I. ABOU-CHAAR for useful discussions.

Test-Tube Fertilization of Ovules in *Melandrium album* Mill. with Pollen Grains of Several Species of the *Caryophyllaceae* Family[1]

The further improvement of the technique of test-tube fertilization may be useful to plant breeders in their efforts to obtain hybrids in those cases where the 2 parents do not cross due to certain obstacles in the path of the pollen tube[2,3]. The present report concerns the development of hybrids derived from the ovules of *Melandrium album* fertilized with pollen grains of 5 different species.

Female flower buds, from which ovules were to be obtained for culture work, were bagged 4 days before pollination. Pistils with a short peduncle were sterilized in saturated chlorine water for 20 min and then rinsed 4 times with autoclaved water. Later the style and the ovary wall were removed and the ovules along with the placenta were inoculated on the medium consisting of WHITE's minerals[4], WHITE's vitamines[4] and 2% sucrose.

Anthers of the flower buds still closed (24 h before anthesis) were excised and kept for 2–4 h in the sterile inoculation chamber. Later the pollen grains were scooped out and dusted on the surface of the ovules. Anthers from the following species have been used: *Melandrium album*, *M. rubrum*, *Silene schafta*, *S. tatarica* and *Dianthus carthusianorum*.

The pollen grains of all species started to germinate within 8–12 h and later on the pollen tubes were entering the micropyle (Figure 1). During the first 3 days of culture the fertilized ovules enlarged, became turgid and in another 7 days they turned white (Figure 2). Dissections of seeds from 14-day-old cultures revealed normally differentiated embryos (Figure 3) and a perisperm fully packed with starch grains. When pollen grains of *Melandrium album*, *M. rubrum* and *Silene schafta* were used, mature embryos were dissected and cultured on a fresh medium. They started to germinate after 2 days and healthy seedlings were obtained by the fourth week (Figure 4). The 10-week-old seedlings, after thorough washing in water, were transferred to soil in pots and raised in the culture room. After another 12–15 weeks, regular flowers had been produced (Figures 5 and 6). Thus fully formed plants with flowers were obtained.

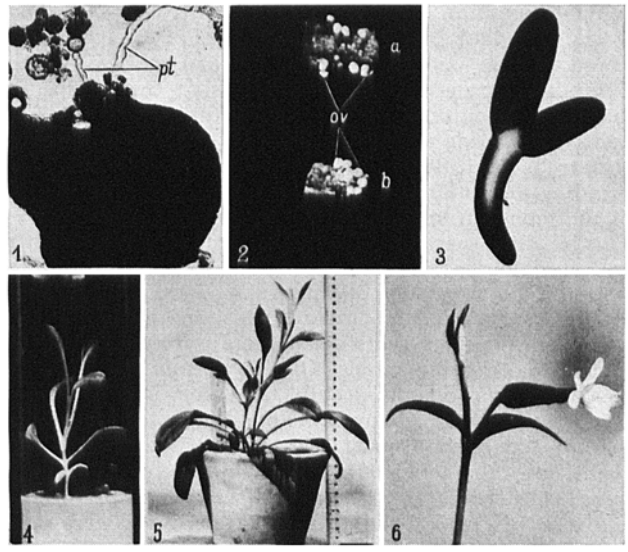

Fig. 1. Whole mount of ovule from 1-day-old culture; 2 pollen tubes (pt) are seen at the micropyle. Pollen grains of Silene schafta. × 50.
Fig. 2. 10-day-old culture; developing ovules (ov) are situated on the placenta. (a) ovules fertilized with pollen grains of *Melandrium rubrum*. (b) ovules fertilized with pollen grains of *Dianthus carthusianorum*. ×1,2.
Fig. 3. Whole mount of an embryo dissected from an ovule 14 days after culturing. Ovules fertilized with pollen grains of *Melandrium rubrum*. × 14.
Fig. 4. A hybrid seedling obtained from a test-tube embryo (ovules were fertilized with pollen grains of *Silene schafta*) after 4 weeks of culture. $^1/_2$ natural size.
Fig. 5. A hybrid plant (*Melandrium* × *Silene schafta*) after 10 weeks of growth in a soil where initially they were transferred from the culture tubes. $^1/_{16}$ natural size.
Fig. 6. A fully developed female flower from a hybrid plant (*M. album* × *S. schafta*) after 12 weeks of growth. $^1/_2$ natural size.

[1] Dedicated to the memory of the late Professor P. Maheshwari who taught me and inspired me to undertake this work.

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