ploïdes sont fréquemment anormales (perte ou non-disjonction de chromosomes). L'un de nous a déjà signalé la présence dans l'épiderme, chez certaines larves aneuploïdes, de mitoses ségrégatives accentuant le degré d'aneuploïdie initial[4,5]. Il ne semble pas, cependant, que ces mitoses soient également fréquentes dans les diverses pontes et ici encore l'hérédité maternelle paraît jouer un rôle déterminant dans la fréquence de leur apparition (BEETSCHEN, FAURIE et LE ROUX, inédit). Nous n'en avons observé que très rarement dans les tissus des embryons appartenant aux deux pontes étudiées en détail.

Les nucléoles des noyaux interphasiques ont une morphologie souvent altérée; ils sont fréquemment vacuolaires et hétérogènes. La synthèse de l'ARN y est sans doute perturbée par l'aneuploïdie. D'autre part on peut observer dans certains cas l'extrusion de nucléoles entiers dans le cytoplasme, où ils dégénèrent probablement.

Summary. A comparison is made between aneuploid offsprings of triploid females mated with diploid or triploid males of *Pleurodeles waltlii*. In the latter case, embryonic lethality is raised, and abnormalities are more severe. The disturbances affect morphogenesis, embryonic growth and physiological differentiation. At the cellular level, nuclear and nucleolar degenerative changes are conspicuous.

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Histochemical Localisation of β-Glucuronidase in Healing Wounds of the Natterjack Toad *Bufo calamita*

It is a well-known fact that β-glucuronidase is related to cell proliferation and the formation of connective-tissue ground-substance. This enzyme has been demonstrated in the basal layers of the oral mucous membrane and the epidermis, the blood vessels of the normal corium, in healing wounds of the skin of the back, the tongue and the palate of the rat[1-3], and in healing wounds of the dorsal skin and the tongue of the common iguana and the axolotl[4]. In continuation of this investigation we have examined the activity of β-glucuronidase in healing wounds of the dorsal skin and the tongue of the toad *Bufo calamita*, the animals being killed after 4, 8, 15, 22, 32 and 40 days. After excision of the wound with an extensive zone of normal surrounding tissue and fixation in formalin/chloral hydrate, the enzymatic activity was determined by the method of FISHMAN and BAKER[5] on frozen sections with different incubation times.

In the superficial necrotic band of the connective tissue in the 4-day specimens a negative reaction was found, whereas immediately beneath this negative zone there was an intensely positive zone due to the presence of inflammatory exudate, and particularly leucocytes.

As healing of the wounds progressed, the fibroblastic proliferation zone reacted moderately but consistently. The histiocytes and giant cells appeared to react more intensely. In the 22-day specimens the epidermis, which had proliferated over the margins of the wound, exhibited a significant increase in enzymatic activity.

In the light of these results we conclude that epithelial proliferations is closely related to β-glucuronidase activity. In connective-tissue this enzyme is associated with tissue formation. As these results correspond with those obtained in the rat[1], the common iguana and the axolotl[4], more extensive significance must no doubt be attached to the β-glucuronidase.

Zusammenfassung. Histochemischer Nachweis von β-Glukuronidase bei der Wundheilung der Kröte *Bufo calamita*.

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The Biogenesis of Helminthosporal

The isolation[1] and elucidation of the structure[2] of the fungal toxin helminthosporal (1) from *Helminthosporium sativum* has recently been reported. This toxin, which is responsible for considerable losses in cereal production, is a sesquiterpenoid, but the structure, though derived from three isoprene units, cannot be formed by the cyclisation of a farnesyl type precursor. This phenomenon, although comparatively uncommon, is not unknown and led RUZICKA to propose the biogenetic isoprene rule wherein the derivation from a farnesyl precursor may be followed by subsequent, and consequently biogenetically trivial, chemical steps[3]. The classic instance of this, is eremophilone, a concluding biogenetic step being a methyl migration. Another mode of transformation from a farnesyl precursor in the sesquiterpenoids is found in the genesis of elemol, where a Cope rearrangement has been proposed[4].

Neither of these schemes appears to be relevant to the biogenesis of helminthosporal. Inspection of the structure suggested that the substance might be derived from a precursor such as (2) (itself derivable from farnesyl cycli-

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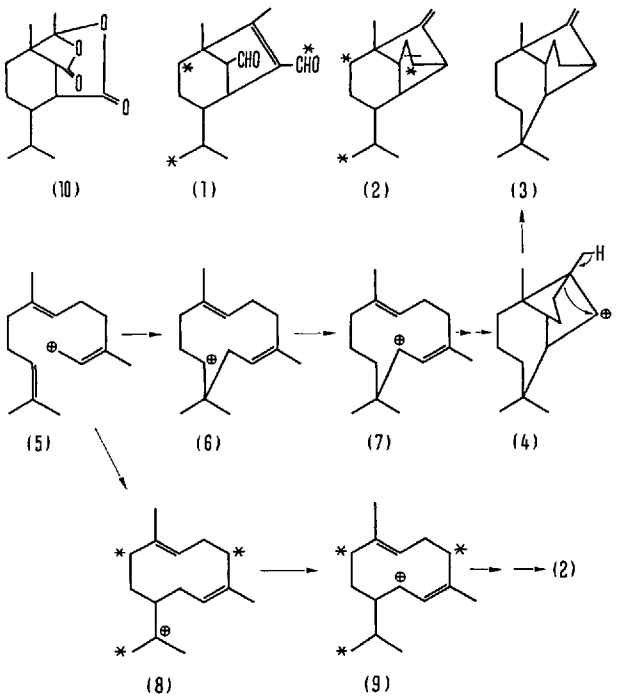

sation as discussed below) by oxidative cleavage at the point indicated. Cleavage of this type has already been suggested in monoterpenoids[5].

If such were the case the unsaturated aldehyde carbon (1, asterisked) should be derived from C_2 of a mevalonic acid unit and not from the 3′ methyl. The structure of this intermediate (2) resembles that of longifolene (3) the biogenesis of which has also not been elucidated. It should be noted that, if the proposal of HENDRICKSON is correct[4], the final stage in longifolene genesis (5) → (6) → (7) → (4) → (3) is a Wagner-Meerwein rearrangement (4, see arrows). However, even if a parallel transformation should occur in helminthosporal genesis the predictions with regards the aldehyde labelling would still be valid.

Helminthosporium sativum was cultured as previously described[1], *DL*-mevalonic acid labelled with carbon-14 at C_2 (100 μC) being added, in methanol, after 3 days growth. The dialdehyde was isolated after 7 days, and converted directly to the stable monocarboxylic acid (1, carboxyl for unasterisked aldehyde) which was crystallised to constant activity (which, after dilution with inactive acid, was 1.88×10^{-4} μC mg[6]) and melting point. Ozonolysis then gave the dilactone (10)[2] in which only the asterisked aldehyde in (1) is lost. This material, also crystallised to constant m.p., had 62% of the original activity, in good agreement with the proposed loss of one of the three radioactive carbons.

If helminthosporal follows a similar pathway to that proposed for longifolene by HENDRICKSON the ion (8)[3,4] must be converted into (9) the subsequent steps being parallel to those for longifolene. No direct evidence of the possibility of such a transformation is available, but it is noteworthy that a number of sesquiterpenoids are known, in which cyclisation evidently proceeds with cationic attack on the isopropylidene group, which do not have the expected double-bond equivalent (derived from the tertiary cation) in the isopropyl side chain. In these cases an oxygen function is present, however, at the α-position. These include carotol[7], acorone[8], junenol[9] and the verbesinols[10].

Transfer by two successive 1:2 shifts or through cyclopropane intermediates are both conceivable[11]. The Hendrickson scheme for longifolene has very recently received support[12].

Zusammenfassung. Die Biogenese des Pilzstoffes Helminthosporal wurde untersucht, indem der Pilz bei Anwesenheit von Mevalonsäure, mit C_2 mit Kohlenstoff-14 markiert, gezüchtet wurde. Es wird gezeigt, dass das Dialdehyd aus einem tricyclischen Vorläufer durch oxidative Spaltung entsteht.

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Distribution of Tritium in WILZBACH Treated Cholesterol[1]

Biological experiments requiring comparatively large amounts of radioactive material suggested the preparation of tritiated cholesterol according to WILZBACH[2]. However, since NYSTROM and SUNKO[3] have shown that cholesterol-H^3 prepared in this manner is contaminated with small amounts of cholestane-3-ol-H^3 by addition of tritium to the double bond, the elimination of this impurity became necessary. This procedure could be carried out indeed as indicated below.

The availability of pure cholesterol-H^3 seemed to make worthwhile an investigation of the distribution of tritium in this molecule especially in view of the interesting results

[1] This work was supported by an Institutional Grant No. P-108A of the American Cancer Society and by a Grant No. G-7477 of the National Science Foundation. The authors are grateful to Dr. M. GUT of this Institute for the WILZBACH treatment of cholesterol.
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