and then the whole culture broth was exhaustively extracted with chloroform. The extract, after having been concentrated to a small volume under reduced pressure, was poured into petroleum ether in order to precipitate the steroid compound. The crude material was purified by column chromatography on florisil and recrystallized from ethyl acetate. The product had the following characteristics: m.p. 196–198°C; $[\alpha]_D + 67.4°$ (dioxane), Anal. calcd. for $C_{22}H_{28}O_5$: C, 70.95; H, 7.58; O, 21.48. Found: C, 70.70; H, 7.66; O, 21.25; λ_{max}^{MeOH} 238 mμ, lg ε = 4.15; IR spectrum (chloroform): 3600 and 3450 cm^{-1} (ν OH), 1702 cm^{-1} (ν C_{20}=O), 1660 cm^{-1} (ν C_3=O), 1620 and 1610 (sh) cm^{-1}) (ν C=C of Δ_{1-4} group), 997 cm^{-1} (considered characteristic for 9α,11α-epoxy configuration), 885 cm^{-1} (CH out of plane deformation of Δ_{1-4} group). For further identification this compound was acetylated in the position 21 with acetic anhydride in pyridine, thus having the following characteristics: m.p. 198–200°C; $[\alpha]_D + 81°$ (dioxane); Anal. calcd. for $C_{24}H_{30}O_6$: C, 69.54; H, 7.29; O, 23.16. Found: C, 69.84; H, 7.52; O, 23.41; λ_{max}^{MeOH} 238 mμ, lg ε = 4.2. This product was identical with a synthetic sample of 16β-methyl-Δ1,4-pregnadiene-9α,11α-epoxy-17α,21-diol-3,20-dione-21-acetate obtained by treatment of 16β-methyl-Δ1,4,9(11)-pregnatriene-17α,21-diol-3,20-dione-21-acetate with perbenzoic acid[3].

The small quantities of a by-product occasionally appearing in some fermentations were revealed by thin layer chromatography on silica gel. In order to isolate this unknown product, the crude material was acetylated in the position 21 and subjected to column chromatography on florisil using chloroform-2% methanol as eluent. The first fractions yielded a substance which was recrystallized from ethyl acetate: m.p. 195–197°C; $[\alpha]_D + 87.9°$ (dioxane), Anal. calcd. for $C_{24}H_{32}O_6$: C, 69.20; H, 7.74; O, 23.05. Found: C, 68.50; H, 7.44; O, 23.65; λ_{max}^{MeOH} 238 mμ, lg ε = 4.1; IR-spectrum (chloroform): 3480 cm^{-1} (ν OH), 1748 cm^{-1} (ν C=O acetate), 1733 cm^{-1} (ν C_{20}=O), 1670 cm^{-1} (ν C_3=O), 1620 cm^{-1} (ν C_4=C_5), 998 cm^{-1} (9α,11α epoxy configuration). The IR-spectrum revealed the presence of the band at 998 cm^{-1} and the absence of the one at 885 cm^{-1}. The compound was different from a synthetic sample of 16β-methyl-Δ1-pregnene-9α,11α-epoxy-17α,21-diol-3,20-dione-21-acetate. From all these data we attributed the double bond to the position 4 and conclude that this by-product is 16β-methyl-Δ4-pregnene-9α,11α-epoxy-17α-21-diol-3,20-dione.

Zusammenfassung. Während unserer Untersuchungen über die mikrobiologische Dehydrierung verschiedener 16β-Methylsteroide durch Bakterienkulturen von *Corynebacterium simplex*, wurde eine neue Art von Umwandlung beobachtet. Von 16β-Methyl-Δ9(11)-pregnen-17α,21-diol-3,20-dion-21-acetat ausgehend, gelang es aus den Kulturfiltraten 16β-Methyl-Δ1,4-pregnadien-9α,11α-epoxy-17α,21-diol-3,20-dion zu isolieren. In einigen Fällen wurde auch das Δ4-Derivat dieser Verbindung als Nebenprodukt erhalten[4].

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A Calcifying Topical Reaction to Mast Cell Depletors in the Rat[1]

Subcutaneous injection of certain mineral salts (e.g. lead, cadmium salts) to the rat results in massive local calcification of dermal collagen fibres[2]. Recently, several compounds listed in the Table were found to elicit a different type of local calcification with predominant involvement of striated muscle fibres at their subcutaneous injection site[3] (Figure 1). These calcifying compounds share the additional properties of being complex organic bases, mast cell depletors[4] and antiheparins as well[5]. The muscular calcification is largely dose-dependent and its intensity independent of an occasional cutaneous necrosis. All other tested mast cell depletors (e.g. dextran, histamine, serotonin, albumen, arginine) produced no such calcifying reaction even though some of them be complex bases.

For histogenetic study, female Sprague-Dawley rats (90–100 g body weight), kept on 'Purina Laboratory Chow' and tap water, were subcutaneously injected in the back with a single dose of polymyxin B sulfate (1.5 mg in 0.2 ml of distilled water) and killed in pairs with chloroform inhalation at various intervals thereafter. Skin specimens at injection site were carefully dissected so as to prevent traumatic disruption of the mast cells, immediately fixed in alcohol-formol (4 parts of absolute alcohol and 1 part of neutral formaldehyde) and embedded in paraffin; sections were stained with a von Kóssa-Azure A procedure for the combined demonstration of calcium phosphates and metachromatic material.

1 h after injection with polymyxin, calcium phosphates were demonstrable in most mesenchymal cells (probably 'activated' fibroblasts), first in their cytoplasm then in and around their nuclei (Figure 2A). After 5 h, liberated hypodermal mast cell granules began to calcify. After 24 h, cutaneous muscle (Figure 2B) and subcutaneous nerves (Figure 2C) showed heavy deposition of calcium. The dermal connective fibres were not visibly calcified. All six

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(Table) calcifying mast cell depletors produced an identical histologic picture.

In rat skin, two distinct groups of substances accordingly appear to elicit local attraction and deposition of calcium, mineral salts in dermal connective fibres, and mast cell depletors mostly in cutaneous muscle fibres and nerves. In addition, certain antiseptics known to be clinically neurotoxic (e.g. stilbamidine, pentamidine) through a disputed mechanism[6] appear to cause local neural damage and calcification, indicating a rather selective neurotropism of these substances. Finally, this calcifying reaction provides one with a simple tool for studying the previously suggested[7] relationship of mast cells with certain types of so-called dystrophic calcification. Factors governing the attraction of calcium and its preferential distribution are under study.

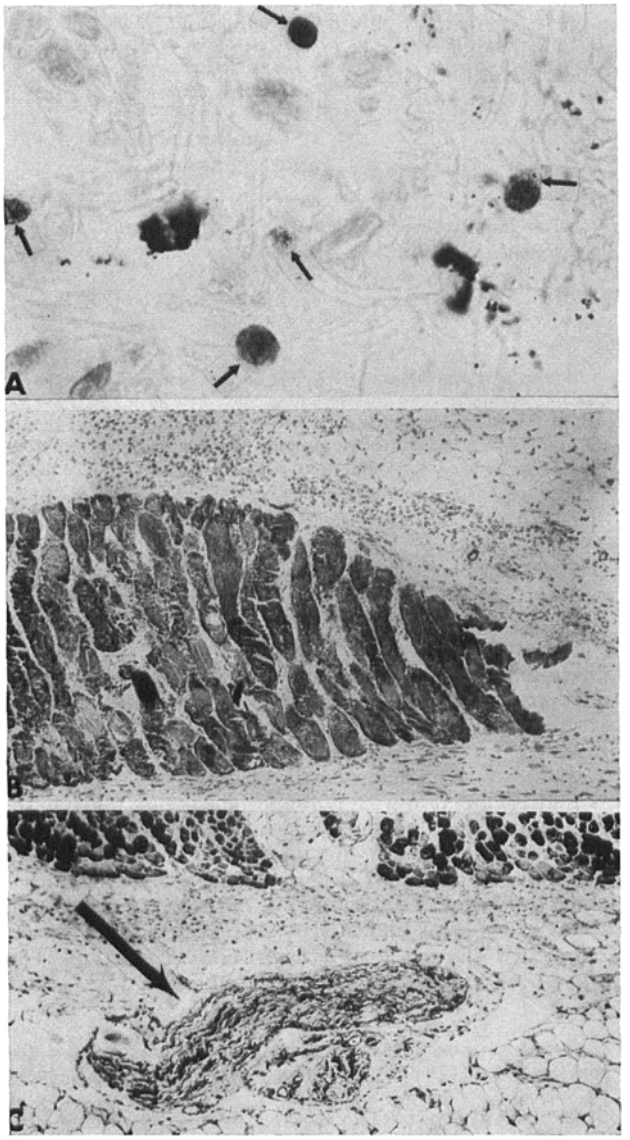

Fig. 2. Histogenesis of the polymyxin-induced calcification (von Kóssa-Azure A). A, 1 h after injection with polymyxin, calcified mesenchymal cells (arrows) around two disrupted mast cells (× 1000). B, 24 h after injection, uniformly calcified dermal muscle fibres, tangentially sectioned (× 120). C, 24 h after injection, heavily calcified subcutaneous nerve (arrow) (× 120).

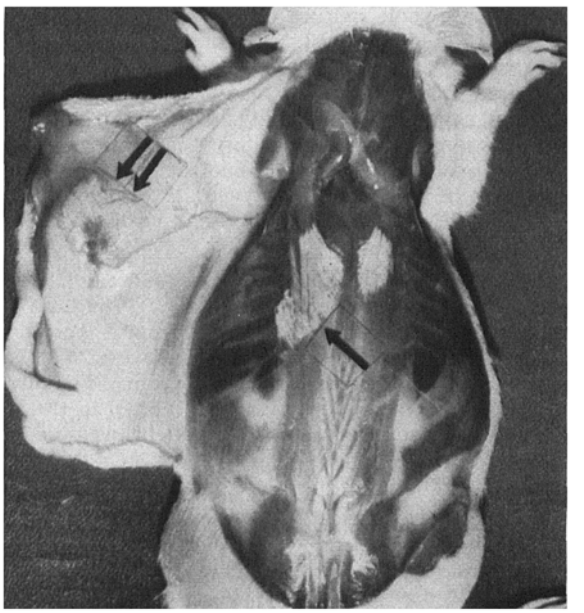

Fig. 1. Calcification of dorsal (arrow) and cutaneous (double arrow) muscles, at the site of injection with polymyxin.

Compound	Source	Range of calcifying dose[a]
Polymyxin B sulfate	Pfizer	1–2
Protamine (Salmin sulfate)	Connaught Laboratories	12–16
Stilbamidine	Delta Chemical Works	3–6
Pentamidine	Delta Chemical Works	1.5–3
Compound 48/80	Burroughs, Wellcome & Co.	0.6–1
Hexadimethrine bromide ('Polybrene')	Abbott	1.5–3

[a] mg in 0.2 ml of distilled water in a 100 g rat. The dose range is the dose producing a limited to extensive calcification.

Résumé. Une réaction de calcification est décrite chez le Rat albinos à l'endroit sous-cutané d'injection de certains dégranulateurs de mastocytes (ex: 48/80). La calcification intéresse surtout le muscle peaucier et les nerfs sous-cutanés. L'accent est porté sur le neurotropisme de ces substances et sur la simplicité de la réaction permettant une étude aisée des relations entre mastocytes et calcification dite dystrophique.

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