Effects of secondary metabolites[a] of *F. oxysporum* f. sp. *carthami* on safflower seedlings

Symptoms due to natural infection recorded at: a) Cotyledonary stage upto first leaf b) 2 weeks after manifestation of infection	Similar symptoms shown by the 3 extractives: $CHCl_3$ (1), EtOAc (2), *n*-BuOH (3) and the culture filtrate (4)[b]
a) Yellowish discolouration at the collar region which later turns brown	4
Blackening of tap roots	2, 3, 4
Shrievelling and crinkling of leaves	3, 4
Bending of leaf lamina on midrib	1, 2, 4
Rolling of leaves	3, 4
Brown necrotic dots on leaves	1, 2, 4
b) Chlorosis	1, 2
Scorching of lamina (necrotic patches)	1
Epinasty	2
Bending of leaf lamina on midrib	1, 2
Flacidity	1, 2
Browning of vascular strands	1

[a] At both concentrations (high and low) similar symptoms appeared; in case of low concentration, the onset of action was delayed. [b] Effect of the culture filtrate was determined only on cotyledonary seedlings.

was dissolved in methanol and the solution was concentrated when lycomarasmin separated as microcrystals (66 mg), m.p. 225–228° (lit.[7] m.p. 227–229°). Hydrolysis of this compound with 1 *N* aq. HCl, on a steam bath for 6 h furnished glycine and aspartic acid.

Assays were conducted with the 3 extractives at ordinary temperature and humidity. Two concentrations of each extractive ($CHCl_3$ extractive, 0.028 µg/ml and 0.28 µg/ml; EtOAc, 0.88 µg/ml, 8.8 µg/ml; *n*-BuOH, 0.52 µg/ml, 5.2 µg/ml) were prepared using Hoagland's solution for dilution. Each of these solutions (20 ml) was taken in a culture tube wrapped with black paper into which 1 disease-free seedling of safflower was introduced. Only the roots were kept immersed in solution. Hoagland's solution was used as control. In another experiment, each of the above solutions (1 ml) was injected to the plant system at the collar region. The results are recorded in the Table. It would seem from the results that the phytotoxic activity of the fungus is not due to a single entity but due to additive effects of the secondary metabolites produced in vitro.

The fact that the fungus is capable of producing highly toxic substances under not very critical conditions, carries with it the possibility that the substances can be produced in the host tissues after the infection. It has been demonstrated here for the first time that trichothecenes, which are known to cause high mammalian toxicity[4], can be translocated in the host tissues from the roots. This study has also established, dispelling earlier doubts, that diacetoxyscirpenol is indeed a vivotoxin. In order to prove this, safflower plants were grown on sterilized soil infected with the fungus. After 60 days, when distinct disease symptoms appeared on the leaf, stem and roots, the plants were harvested. The individual parts were washed and then macerated with water and chloroform in a high speed blender. The chloroform extracts were processed in the usual way. The residue showed the presence of diacetoxyscirpenol and T-2 toxin by its fluorescence under UV-light (short wavelength) on TLC, development of purple and violet colours with Ehrlich reagent, and by the capacity to kill apical buds of safflower which was followed by the appearance of new shoots of auxilliary buds. This last effect is very similar to that reported[8] for diacetoxyscirpenol on winter tares. The amounts of the trichothecene derivatives were maximum in the roots and minimum in the leaves. The translocation of the trichothecene derivatives was demonstrated by their movement through conductive tissues when the total chloroform extractive was injected or when it was soaked in the root system. On one occasion, even when the mycelium was absent, diacetoxyscirpenol was isolated from seeds of safflower.

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β-Blockade of Morphine-Induced Hyperlactacidemia in Rabbits[1]

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Summary. In morphinized rabbits blood lactate levels are elevated. Hyperlactacidemia persists after cessation of morphine injections. This morphine-induced lactate accumulation is completely abolished by simultaneous propranolol treatment. Phentolamine does not modify the action of morphine.

In morphinized animals, blood and various other tissues have elevated lactate levels[3,4]. This indicates a profound change in cellular metabolism which might be related to the characteristic disturbances of the abstinence syndrome. The mechanism responsible for the hyperlactacidemia following morphine administration is not yet clear. It is possible that it results partially from catecholamine secretion, at least for the first few injections. We have tried to prevent the lactate accumulation which occurs in morphinized rabbits by pretreating the animals with adrenoblocking agents: phentolamine and propranolol.

Materials and methods. Male Fauve de Bourgogne rabbits weighing 2.5 kg were used. Blood samples were taken from the marginal vein of the ear at 09.00 h in 15 h-fasted animals. Morphine hydrochloride (Chaix and du

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Table I. Effect of the adrenergic blocker, phentolamine (1 mg/kg, i.v.), on blood lactate in normal and morphinized rabbits

Lactate (mg/100 ml plasma)					
Time	0	30 min	1 h	3 h	6 h
Phentolamine (6)	14.6 ± 1.6	19.5 ± 4.9	16.2 ± 4.5	12.7 ± 2.0	14.9 ± 1.4
Morphine (14)	15.7 ± 1.8		42.7 ± 6.2	39.4 ± 4.5	22.4 ± 3.4
			$p < 0.001$	$p < 0.001$	NS
Morphine + phentolamine (6)	11.6 ± 1.3	16.0 ± 0.1	37.5 ± 4.9	41.5 ± 4.9	20.6 ± 2.3
		NS	$p < 0.001$	$p < 0.001$	$p < 0.05$

Values are means ± SE. The number of animals is given in parentheses. The significance was determined by the Student's t-test vs 0 values.

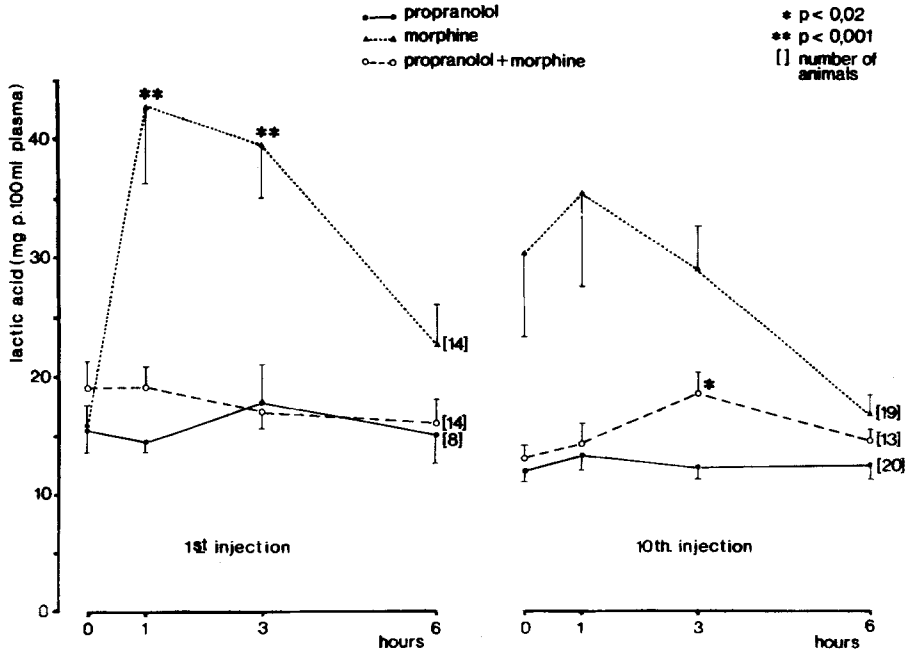
Marais) was administered i.m. at a dose of 10 mg/kg. Phentolamine (Regitine Ciba Geigy) was administered (1 mg/kg i.v.) as an α-blocker and propranolol (ICI) was used as β-blocker (5 mg/kg i.p.). These agents were injected alone or in association with morphine. Lactate was measured by the method of BARKER and SUMMERSON[5].

Results. Effects of phentolamine: When injected alone at a dose of 1 mg/kg i.v., phentolamine has no significant effects on circulating lactate levels in rabbits (Table I). When administered in association with morphine, phentolamine does not suppress the morphine-induced hyperlactacidemia.

Effects of propranolol: Results are summarized in the Figure. When administered alone (5 mg/kg) to fasting animals, propranolol has no significant effect on circulating lactate. Morphine alone (10 mg/kg) causes a very clear rise in blood lactate. This effect is completely suppressed in animals pre-treated with the β-blocker.

When animals are chronically (at least 8 days) treated with daily injections of propranolol (5 mg/kg), of morphine (10 mg/kg) or both substances, propranolol still has no effect. In addition, basal lactate levels in chronically morphinized animals are much higher than in normal animals (30.3 ± 6.9 versus 15.7 ± 1.8 mg per 100 ml; $p < 0.02$). Simultaneous daily treatment with propranolol completely suppresses the lactate accumulation which is normally present during morphine intoxication. Tolerance had developed towards the initial effect of morphine since lactate levels in rabbits treated for 8 days were not significantly changed by a new injection. Complementary experiments done with fed animals show that fasting substantially lowers circulating lactate levels, but that this nutritional effect disappears during chronic treatment with propranolol (Table II).

Discussion. The present work shows that the effects of morphine on blood lactate in rabbits are similar to those previously described in rats[3,4] and morphine-induced lactate accumulation is completely abolished by simultaneous propranolol treatment. It is well known that hyperlactacidemia usually results from cellular hypoxia or from adrenaline release, the causes of which are very diverse. The major lactate producing tissue is muscle. In rats, the effect of adrenaline on muscle is mediated by β-receptors[6,7]. The role of β-receptors in adrenaline-induced glycogenolysis is well established in muscle, whereas great discrepancies exist between results concerning the nature of receptors in hepatic tissue. Numerous authors have reported propranolol blockade of adrenaline-induced glycogenolysis in muscle [8,9]. Our results show that propranolol prevents the excess lactate production which occurs in morphinized rabbits. This



Changes in blood lactic acid induced by morphine, propranolol and propranolol + morphine. The standard errors are shown by the vertical lines. The number of animals is indicated between brackets.

suggests that the morphine-induced hyperlactacidemia results largely from anerobic muscle glycogenolysis which is mediated by β-adrenergic receptors. On the other hand, it seems that α-receptors are not involved since phentolamine has no effect of its own nor does it modify the action of morphine.

Hyperlactacidemia is a constant metabolic symptom in morphinized animals, even more interesting as it persists after cessation of morphine treatment. In our experimental series blood lactate concentration remains significantly higher than control level up to 10 days after stopping an 8-day morphine treatment. Given the permeability of the blood brain barrier to lactate[10], an accumulation of lactate in the blood can result in a higher concentration in the brain as has been shown in morphinized rats[3]. Abnormally high brain lactate concentration might be related to the state of anxiety[11] found in drug addicts under withdrawal. We have shown that hyperlactacidemia persists after withdrawal from morphine and that it is completely suppressed by propranolol. This is very interesting because of recent reports demonstrating the effectiveness of propranolol in treating anxiety in man[12,13] and heroin addicts[14].

Table II. Effect of propranolol (5 mg/kg) on blood lactate in fed (N) and 15 h fasted (F) rabbits

Lactate (mg p. 100 ml plasma)				
Time	0	1	3	6 hours
1st injection				
F (8)	15.6 ± 1.9[1]	14.6 ± 1.1	17.7 ± 3.2	15.0 ± 2.4
N (8)	34.1 ± 5.3[2] $p < 0.01$ [a]	29.3 ± 3.8	29.7 ± 4.4	28.5 ± 3.4
10th injection				
F (8)	14.4 ± 0.9	16.9 ± 2.0	14.3 ± 1.1	16.4 ± 1.9
N (8)	16.4 ± 2.3 $p < 0.01$ [b]	17.2 ± 0.8	17.4 ± 2.4	20.3 ± 2.1

Values are means $\pm$ SE; p is given by Student's t-test. [a] vs (1); [b] vs (2). The number in parentheses represent the number of animals.

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Frescon: Neurophysiological Action of a Molluscicide

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Summary. The molluscicide N-trityl morpholine ('Frescon') has an unusual effect on the central nervous system of a freshwater snail. Nerve impulses become grouped into spontaneous 'bursts', with many cells firing synchronously. This may result from interference with inhibitory processes.

N-tritylmorpholine (Frescon, Shell Chemicals) is toxic to many freshwater snails[3]. It is highly specific, being harmless to plants, insects and most vertebrates, though it is moderately toxic to some species of fish[4]; even the terrestrial Gastropod *Helix aspersa* is apparently immune[4]. It is therefore important in controlling the snail hosts of a number of parasites, including species of *Schistosoma* which give rise in man to the widespread tropical disease, bilharzia[5].

Frescon is lethal to freshwater pulmonate Gastropods at very low concentrations (e.g., *Biomphalaria glabrata*: LD_{50} (24 h) $= 2.5 \times 10^{-8}$ g/ml[6]). *Lymnaea stagnalis* is killed by 10^{-6} g/ml Frescon in 3 h at 20 °C. Its specificity and rapid effect have caused speculation as to its mode of action.

Investigations so far have not suggested any interference in such metabolic processes as oxidative phosphorylation[4]. Preliminary experiments have however suggested that Frescon may cause abnormalities in the electrophysiology of the *Lymnaea stagnalis* nervous system[4]. To investigate this further, individual nerve cells from the visceral or right parietal ganglia of the isolated central nervous system of this species were impaled by 1 M potassium acetate microelectrodes (ca. 25 $M\Omega$) using standard electrophysiological recording techniques. Separate recording and stimulating electrodes were used to allow control of membrane potentials. Normal Ringer solution[7] consisted of 50 mM NaCl, 1.6 mM KCl, 4 mM $CaCl_2$, 2 mM $MgCl_2$ and 5 mM *Tris*-Cl (pH 8.0) in distilled water. In more recent experiments, 50 mM sucrose was also added to provide a better osmotic balance. Low Ca/high Mg Ringer contained 2 mM $CaCl_2$ and 20 mM $MgCl_2$, with 23 mM sucrose to maintain overall osmolarity. As Frescon is very hydrophobic, it was used in a di-

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