then go through a specific sequence of differentiation before tympanal cuticle is produced. This hypothesis would be consistent with the presence of the depressed areas and reduced density of sensilla in the tympanal region of regenerates following amputation at 7th instar. During normal development the tympanal areas gradually lose their sensilla over the last 3 immature instars before the distinctive adult tympanal cuticle is produced at the imaginal moult. Another possible explanation of the results is that cells are committed to form tympanal tissue at a certain critical time in the life of the insect. If this time is missed the signal is no longer present or the presumptive tympanal cells are no longer capable of responding.

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The fasciolicidal activity of a halogenated benzenesulfonanilide

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Summary. Halogenated benzenesulfonanilides have potent fasciolicidal activity. The efficacy of 1 such compound in mice, sheep, and cattle is reported.

Certain orthosubstituted halogenated benzenesulfonanilides have been known to possess antimicrobial[2] and molluscicidal[3] activity. Activity against the common liver fluke, *Fasciola hepatica,* has not previously been reported.

Compounds were detected as part of a routine screening program for chemicals with fasciolicidal activity. The screening method has been described[4]. Numerous 2-hydroxy as well as 2-acetoxy and 2-propionoxy substituted benzenesulfonanilides with various halogen constituents have been found[5] to possess marked fasciolicidal activity. Data from studies with a potent compound of this type is presented here to illustrate such activity.

The compound, 2-hydroxy-2′,3,4′,5,5′,6-hexachlorobenzenesulfonanilide, was 100% effective against *F. hepatica* in mice when administered for a 7-day period (14–21 days post infection) in the diet at 0.0125%; an effective concentration that compares very favorably with known fasciolicides[6]. As a single oral dose, the $ED_{>90}$ in mice against 14-day-old flukes was 30 mg/kg and the LD_{50} in normal mice was 179 mg/kg.

Established fasciolicides are known to exhibit different degrees of efficacy depending on the age of the parasite at the time of treatment. Thus in sheep the compound was tested against experimentally induced fascioliasis at 4, 6, and 14 weeks after infection (table 1). Against a 4-week-old *F. hepatica* infection a single oral dose of 5, 10 or 20 mg/kg b.wt was 98, 100, and 100% effective, respectively. Against 6-week-old flukes, 3, 4 and 5 mg/kg were highly effective giving reductions of 96, 99, and 100%, respectively, while 2 mg/kg resulted in an apparent reduction of 42%. Treat-

Table 1. Efficacy of 2-hydroxy-2′,3,4′,5,5′,6-hexachlorobenzenesulfonanilide against experimental *Fasciola hepatica* infections of various durations in sheep.

Experiment	Age of infection at treatment	Dose (mg/kg)	Mean number of live flukes recovered	% Reduction
1	4 weeks	0	154	0
		20	0	100
		10	0	100
		5	3.0	98
2	6 weeks	0	57	0
		5	0	100
		4	0.5	99
		3	2.5	96
		2	33	42
3	14 weeks	0	141	0
		5	0	100
		2	1.5	99
		1	63	55
		0.5	121	14

Experimental conditions: 28 mixed-breed young sheep were used in 3 separate experiments. The animals were approximately 12–14 weeks old when exposed orally to 400 (experiment 1), 100 (experiment 2), or 200 (experiment 3) selected metacercariae of *Fasciola hepatica.* At the time shown for each experiment, the animals (weight ca. 30 kg experiments 1 and 2; 40 kg experiment 3) in groups of 2 were each given a single oral dose of drug suspended in Methocel vehicle (experiments 1–1.75% Methocel, 20 ml/sheep; experiment 2–1.25% Methocel at a volume of 1 ml/kg; experiment 3–1.25% Methocel, 0.5 ml/kg). In each experiment 1 group received vehicle without drug (0 dose group = placebo). The animals were necropsied 13 or 14 days after medication and flukes recovered by dissection of the liver.

$$\% \text{ Reduction} = \frac{\text{placebo count} - \text{test count}}{\text{placebo count}} \times 100$$

ment given at 14 weeks resulted in marked reductions at 2 and 5 mg/kg (99 and 100%) with a loss of marked activity at 1 and 0.5 mg/kg. Toxic effects were observed only in the 20-mg/kg-group of experiment 1. In this case 1 of the 2 sheep was functionally blind 3 days after treatment, but otherwise clinically normal.

In studies in sheep to assess toxicity it has been found that blindness with an ocular pathology characterized by corneal edema, equitorial lens opacities, papilledema, and changes in the retinal vessels, can follow oral administration of the compound at a level above 20 mg/kg. Blindness in some sheep as a sequel to treatment with a single dose of 20 mg/kg has been confirmed; 15 mg/kg was associated with slight papilledema in 1 of 3 sheep, and no definite abnormalities were noted with a treatment of 10 mg/kg.

Table 2. Efficacy of 2-hydroxy-2′,3,4′,5,5′,6-hexachlorobenzenesulfonanilide against mature *Fasciola hepatica* in the bovine

Dose (mg/kg)	Mean number of live flukes recovered	% Reduction
0	137	0
5	2.5	98
4	39	72
3	72	48
2	132	4

Experimental conditions: 10 mixed-breed calves were each exposed orally to 600 selected metacercariae of *Fasciola hepatica.* 14 weeks after exposure the animals (mean weight 186 kg) in groups of 2 were each given a single oral dose as shown of compound suspended in 1.25% Methocel vehicle (approx. 0.15 ml/kg b.wt). 1 group received vehicle only (placebo). 13 days after treatment the calves were killed and flukes recovered by dissection of the liver.

In a limited efficacy trial in cattle (table 2) the compound, given as a single oral dose of 5 mg/kg, was 98% effective against 14-week-old flukes. Doses of 4, 3, or 2 mg/kg gave apparent reductions of 72, 48, and 4%, respectively. No clinical manifestations of toxicity were noted in any group during the trial.

As illustrated in this report, halogenated benzenesulfonanilides are potent fasciolicides with excellent activity against both immature (< 12 weeks old) and mature flukes. In common with certain known fasciolicides[7,8], the chemotherapeutic index was narrow. Alterations in chemical structure, formulation, and method of administration can, however, significantly influence this parameter.

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Seasonal variation of androgen interconversion in testicular tissue of *Rana temporaria* in vitro

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Summary. Testicular steroid metabolism of winter and spring frogs, *Rana temporaria,* were studied by in vitro incubation with radioactively labelled dehydroepiandrosterone and androst-4-ene-3,17-dione. Marked seasonal differences were observed which are in line with the findings of others.

The breeding cycle of frogs is known to be due to an independent inherent rhythm[1]. The spawning is timed to occur just after the end of the wintering period and the behavioural change is believed to be caused by increased hormonal activity. A seasonal variation in plasma levels of testosterone, estradiol and estrone has been found to occur in *Rana esculenta*[2]. Δ^5-3β-Hydroxysteroid dehydrogenase activity has earlier been shown histochemically both in anuran and urodelan testes[3]. This reaction was most intense with dehydroepiandrosterone in *Xenopus* testes[4]. Incubation studies have shown the presence of Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSD), 17α-hydroxylase, C21-C19-desmolase and 17β-hydroxysteroid dehydrogenase (17β-HSD) activities in testes of *Pleurodeles waltlii*[5], *Rana esculenta*[6] and *R. catesbeiana*[7].

We report here seasonal differences in the testicular steroid metabolism of winter (December) and spring (May) frogs, *R. temporaria.* In Southern Finland the spawning starts on the average on 20 April[8].

Materials and methods. 20 adult frogs caught in May in Southern Finland were used immediately (spring frogs). 40 frogs caught in September were kept for a few months in captivity at 4 °C and in the dark. This latter group was used in December (winter frogs). Body and testis weights were determined before incubation. The reproductive condition of the spring frogs was seen by the occurrence of spermatozoa in the vesicula seminalis. To 10 of the winter frogs, 500 IU of HCG (Pregnyl®, Organon) was given per animal a day before the experiment. Pooled testes were homogenized in frog-Ringer buffered by Trizma® (Sigma) solution pH 7.5 at 15 °C to obtain a 10% homogenate which was incubated as 20 5-ml portions at 15 °C for 30 min under a continuous flow of carbogen. 10-min incubation time was also tested and found insufficient for the measurable conversion of the substrate. Substrates were 4-^{14}C-DHA (0.58×10^{-3} mmole/l, 0.15 µCi) and 4-^{14}C-androst-4-ene-3,17-dione(Δ^4A, 0.58×10^{-3} mmole/l, 0.15 µCi). When NAD or NADH was used concentration was 1.51 mmole/l. 6 control incubations were carried out using acetone denatured homogenates and 4-^{14}C-DHA as substrate. Isolation by diethyl ether and characterization of the radioactive metabolites were done as previously described[9]. The meta-