Tab. III. Influence of pretreatment with chlorpromazine on pentobarbital narcose in adrenalectomized[a] or immature[b] rats.

Treatment	No. of animals	Sleeping-time (min) ± S.E.	% variation	p
I Controls	16	18 ± 3·9	—	—
Adrenalectomized rats	14	35 ± 5·0	—	—
Adrenalectomized rats + Chlorpromazine	12	11 ± 3·2	−68	$0{\cdot}01 > p > 0{\cdot}001$
II Immature rats	14	41 ± 2·9	—	—
Immature rats + Chlorpromazine	14	26 ± 2.9	−37	$p < 0.001$

[a] Female rats weighing 180 g, adrenalectomized 7 days before narcose and treated daily with 1 mg/kg cortisone acetate (s. c.). All rats were kept at 25°C room temperature and received 1% NaCl solution, as drinking water. Chlorpromazine was injected at the dose of 15 mg/kg and pentobarbital at the dose of 20 mg/kg i. p.

[b] Female immature rats (30 days old, average body weight 100 g) are used. The treatment dose of chlorpromazine was 10 mg/kg (i. p.) pentobarbital (22 mg/kg) was injected i. p.

It is well known that the barbiturate breakdown is modified by the cortical[2] and sex[3] hormones. For this reason we studied the late effect of chlorpromazine in adrenalectomized and immature rats. Our results are reported in Table III.

From our data it is possible to conclude that chlorpromazine pretreatment is able to reduce pentobarbital sleeping-time after 48 h also in the adrenalectomized and immature rats.

A possible decrease of barbiturate levels in brain, when the sleeping-time was reduced, was also investigated. In experiments injecting 15 mg/kg of chlorpromazine, and 48 h later, 25 mg/kg of pentobarbital, it was observed that the barbiturate concentration in brain of animals killed 1 h after the administration, was only 60% in comparison with that of the controls[4].

Our results can be explained with a decreased penetration of barbiturates into the brain or with an enhanced breakdown. The second effect is more probable. Recently, REMMER[5] observed an increase of hexobarbital oxidation by the liver microsome enzymes in rats pretreated with barbiturates. On the other hand, in very recent experiments, we were able to observe that chlorpromazine pretreatment can decrease the toxicity of strychnine and picrotoxin and the pharmacological effects of meprobamate and myanesin[6].

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Riassunto

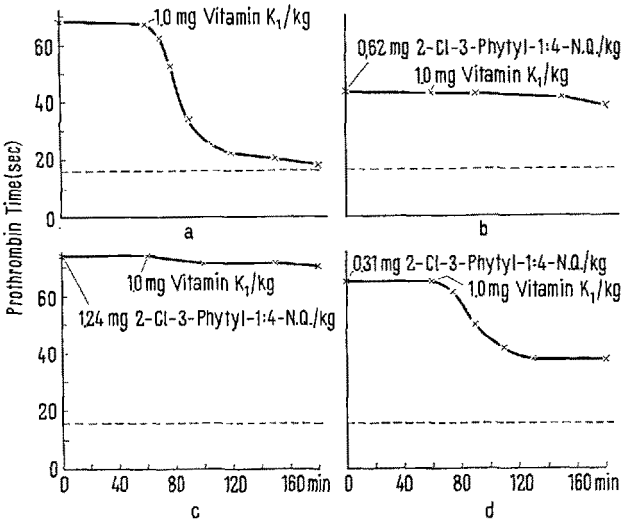
L'autore osserva che un trattamento fatto 48 h prima con cloropromazina induce nei ratti una diminuzione nel tempo di sonno da pentobarbital e da esobarbital, che si accompagna a una diminuzione della concentrazione encefalica dei barbiturici. Il fenomeno non si avvera per il sonno da «Doriden», nè da «Viadril», nè da alcool.

The Inhibition of the Antidotal Activity of Vitamin K_1 against Coumarin Anticoagulant Drugs by its Chloro Analogue[1]

The replacement of a methyl group by a chlorine atom in a biologically active compound may result in an analogue with inhibitory activity. The application of this principle has led to the synthesis of chlorine analogues of riboflavine which are competitive inhibitors[2,3]. Since vitamin K_1, 2-methyl-3-phytyl-1:3-naphthoquinone, contains a methyl group, its chloro analogue, 2-chloro-3-phytyl-1:4-naphthoquinone, has been synthesized and tested for its ability to inhibit the antidotal activity of vitamin K_1 against coumarin anticoagulant drugs.

Rabbits weighing from 2·5 to 3·5 kg were fed Warfarin [3-(α-phenyl-β-acetylethyl)-4-hydroxycoumarin] using a dose of 15 mg/kg of body weight/day for two days. This increased the prothrombin time to approximately 70 sec compared to a value of 15 to 17 sec before treatment. In such animals, the intravenous administration of 1 mg/kg of body weight of vitamin K_1 resulted in a significant reduction of the prothrombin time within 40 to 60 min (Fig. a). When the chloro analogue was administered intravenously 60 min before, 1·24 mg and 0·62 mg/kg completely blocked (Fig. b and c), whereas 0·31 mg/kg significantly reduced the antidotal effect of vitamin K_1 (Fig. d).

The precise site or mode of action of vitamin K is not known. QUICK[4] and ALMQUIST[5] have suggested that certain plasma clotting factors (prothrombin, factors VII, IX, X) are synthesized by an enzyme system of which the vitamin is a coenzyme. Because all compounds with Vitamin K activity are para-quinones, they may function as components of an electron transport system. Coumarin anticoagulaut drugs must interfere with this system by depleting it of the coenzyme, as their effect is reversed readily



a) Vitamin K_1 alone, b) c) d) 1·24 mg, 0·62, 0·31 mg respectively o 2-Chloro-3-Phythl-1:4-Naphthoquinone 60 min before the administration of Vitamin K_1.

[1] This work was supported by a grant from the National Research Council of Canada.

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by vitamin K. The 2-methyl group also is essential for vitamin K-like activity and perhaps serves to anchor the vitamin K molecule to the apoenzyme[6]. While the methyl group and the chlorine atom have similar shapes, they differ in their polar resonance and inductive effects. The replacement of the 2-methyl group by a chlorine atom can result in an analogue which is sufficiently similar to occupy the free receptor sites on the surface of the apoenzyme in the coumarin anticoagulant treated animal but which is unable to elicit the biological effects of vitamin K.

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Zusammenfassung

Die Wirkung von Vitamin K_1 auf mit Coumarin behandelte Kaninchen wird durch 2-Chloro-3-Phytyl-1:4-Naphthochinon gehemmt.

Uptake of ^{35}S Labelled Sulfate in the Exorbital Lacrymal Glands of Adult and Newborn Rats under Different Hormonal Treatment

Previous experiments have shown that testosterone treatment in normal and castrated male rats results in morphological changes of the exorbital lacrymal glands (Loewenthal glands), i. e. appearance of a tubular structure with mucous secretion and increase of the nuclear volumes (Cavallero and Morera[1]). An additional research has been now undertaken in order to show whether or not testosterone, in comparison with other hormones, is capable of affecting the uptake of ^{35}S labelled sulfate by the glands both in adult and newborn rats.

In a first experimental series, adult male castrated rats were used. Four animals without hormonal treatment and four treated with testosterone propionate (1 mg daily subcutaneously for 10 days) were injected intraperitoneally with ^{35}S labelled Na_2SO_4, at a dosage of 0·3 μC/g body weight. 18 h after the injection they were sacrificed, their exorbital lacrymal glands were carefully dissected, weighed, and dissolved in 0·5 cm^3 of conc. hydrochloric acid. The solution was then dried with infrared rays and the radioactivity of the dry material evaluated with a Geiger counter (mica-end-window weighing 2·6 mg/cm^2). The radioactivity was expressed as counts/min/mg wet weight.

From this first experiment, it was found that the ^{35}S-uptake was higher for testosterone-treated glands; the mean count/min/mg was 11 for the control glands, whereas 27 impulses were counted with the testosterone-treated glands, the difference being highly significant.

In a second experimental set, two-days old rats were used, partly untreated and partly treated with various hormones, as reported in the Table. Hormonal treatment lasted three days; thereafter radiosulphate, 1 μC/g of body weight, was injected. Other procedures were identical to those of the first series. The data were analyzed statistically by the analysis of variance technique. Statistical significances were assessed at 1% level.

^{35}S uptake in the exorbital lacrymal glands of 2-days old rats under different hormonal treatment.

No. animals	Treatment	Daily dose	Net counts/ min/mg
8	Controls	—	33
5	Testosterone	0,1 mg	36
5	TTH (Organon)	0·2 mg	43[a]
6	Thyroxine (Roche)	0·01 mg	37
5	Cortisone	0·1 mg	17[a]
4	Insulin	0·1 I.U.	30
4	Glucagon (Lilly)	0·1 mg	20[a]

[a] Differs significantly from control group.

From this Table, it appears that in newborn rats no change occurs in the ^{35}S uptake by the glands after testosterone, thyroxin, and insulin treatment; on the other hand, there is an increase following thyrotropin and a decrease after both cortisone and glucagon treatment. Our results with thyrotropin are similar to those obtained by Wegelius *et al.*[2] on other lacrymal glands (ventral and Harderian glands) of the guinea pig, where hormonal treatment actually increased ^{35}S uptake.

Histological studies on adult Loewenthal glands have shown that testoterone treatment results in important morphological changes; i. e. appearance of a tubular structure with collection of mucous material in the lumina, associated with increased ^{35}S uptake. On the other hand, histological examination of the Loewenthal gland in newborn rats showed evidence of a conspicuous structural immaturity; in addition, the treatment with testosterone did not elicit the structural modifications seen in the adult, nor cause any change in the uptake of ^{35}S.

We can thus conclude that Loewenthal glands of the newborn animal are not sensitive to testosterone action, while being quite responsive to the action of TTH, or, more precisely, to the ophthalmotropic activity of the complex thyrotropic hormone, behaving like the other lacrymal glands. Moreover, there seems to be a close relationship between mucous changes of the glands and increased ^{35}S uptake.

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Zusammenfassung

Die Verfasser weisen nach, dass die Testosteronbehandlung in der äusseren Orbitaldrüse (Nebenohrspeicheldrüse; Loewenthalsche Drüse) der erwachsenen Ratte die Aufnahme von ^{35}S steigert. Gleichzeitig wird eine tubuläre Veränderung der Drüse mit schleimiger Sekretion beobachtet. Diese Erscheinungen sind beim neugeborenen Tier nach Testosteron nicht nachweisbar, hingegen wird hier durch Thyrotropin die ^{35}S-Aufnahme gesteigert.

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