Table II. Average length of time elapsed between the formation of the experimental groups and the moults: cumulative data

Type of moult		No.	Days	Log	P
Larval	Control	15	22.17	1.346 ± 0.079	< 0.05
	Treated	29	39.90	1.517 ± 0.040	
Supplementary reproductives	Control	23	15.53	1.191 ± 0.054	< 0.01
	Treated	90	22.74	1.357 ± 0.027	
Soldiers and intercastes	Control[a]	58[a]	43.09[a]	1.634 ± 0.035	< 0.01
	Treated	155	16.03	1.205 ± 0.016	

[a] Data collected by the author in other researches (only soldiers).

that on the average the moults into supplementary reproductives take place first, followed by larval moults and, simultaneously, moults into soldiers. In the groups treated with FAEE, moults into soldiers and into intercastes are the first to take place, followed by moults into supplementary reproductives and then, last of all, by larval moults. Evidently the substance does not act only on pseudergates that are in the well-defined period of competence for differentiation into soldiers.

The frequency of moults into soldiers and intercastes increases as the dose of FAEE administered to the pseudergates is increased; on the other hand, larval moults and moults into supplementary reproductives diminish; only at very high doses are white soldier obtained. There was an increase in the average time between the beginning of the treatment and the larval ecdyses and ecdyses into supplementary reproductives, whereas the times of ecdysis into intercastes and soldiers became shorter, compared with the times observed in other experiments[8].

Riassunto. Per studiare l'influenza del farnesato di etile (FAEE) sulla differenziazione delle caste, si sono trattate pseudergati di *Kalotermes flavicollis* con dosi differenti di sostanza. Si è ottenuta la differenziazione sia di soldati, sia di intercaste: tra soldato e pseudergate e tra soldato e reale di sostituzione; la frequenza di questi tipi di mute aumenta con la dose di FAEE usata, mentre diminuisce la frequenza delle mute larvali e a reale di sostituzione. Il tempo medio intercorso tra l'inizio del trattamento e le mute larvali o a reale di sostituzione è stato più lungo che per i controlli, è stato invece più breve per le mute a soldato e a intercasta di soldato.

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Antitumor Effect of a New Retinoic Acid Analog

Retinoic acid[1] has been shown to have a prophylactic and a therapeutic effect on chemically induced benign and malignant epithelial tumors in mice (BOLLAG[2–4]). Skin papillomas as well as skin carcinomas of mice induced by Dimethylbenzanthracene and croton oil could be made to regress either partially or completely by systemic administration of retinoic acid. In clinical studies the treatment of actinic keratoses and basal cell carcinomas with local application of retinoic acid led to partial or complete regression of these lesions (BOLLAG and OTT[5]). Further positive therapeutic results were obtained when retinoic acid was administered orally to patients with premalignant conditions of the skin or mucous membranes, e.g. leukoplakias of the mouth, tongue and larynx (RYSSEL et al.[6]). Papillomas of the urinary bladder, too, have been influenced favourably by the oral administration of retinoic acid (EVARD and BOLLAG[7]). Although from a scientific point of view interesting results have been achieved, this treatment cannot be recommended for practical purposes because of side effects. Retinoic acid induces, in animals as well as in man, a series of toxic effects, well known under the name of the hypervitaminosis A syndrome. The main symptoms in man are headache and alterations of the skin and mucous membranes. In small rodents bone fractures are a prominent feature. These side effects limit higher and therapeutically more efficacious dosages. Our aim was therefore to find derivatives with a better therapeutic ratio, possessing a more favourable relation between the tumor-active dose and the hypervitaminosis A causing dose. Among a large series of retinoic acid analogs, synthesized by RUEGG and RYSER in the Roche Laboratories[8], the aromatic analog all-trans-9-(4-methoxy-2, 3, 6-trimethylphenyl)-3, 7-dimethyl-2, 4, 6, 8-nonatetraenoic acid, as well as its esters and amides, proved to be particularly active preparations. The following investigations have been carried out with the ethyl ester I (Figure) of the above-mentioned free acid.

[1] Retinoic acid = all-trans-β-retinoic acid = vitamin A acid = Retinsäure.

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Table I. Results of treatment of skin papillomas for 2 weeks with retinoic acid and its analog I

Dose (mg/kg)	Average sum of the papilloma diameters per animal (mm)		Change in the average sum of the papilloma diameters per animal (%)
	Day 0	Day 14	
Controls	21.1	26.0	+23.2
Retinoic acid			
400 1× weekly i.p.	21.6	11.2	−48.2
200	24.7	15.8	−36.0
Analog I			
200 1× weekly i.p.	25.5	6.6	−74.1
100	28.0	8.5	−69.6
50	20.4	9.1	−55.4
25	24.4	12.5	−48.8
12.5	24.8	17.3	−30.2
Analog I			
400 1× weekly p.o.	22.0	4.8	−78.2
200	23.7	9.8	−58.7
100	21.5	14.3	−33.5
50	21.0	16.5	−21.4
25	16.0	14.2	−11.3

Comparing the average sum of diameters on day 0 and day 14 the t-test showed significant decreases ($P < 0.05$).

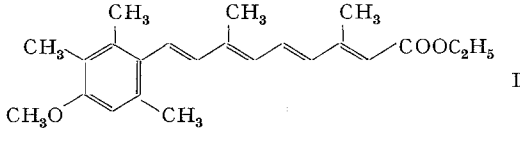
Table II. Results of treatment of skin carcinomas for 2 weeks with the retinoic acid analog I

	Mean carcinoma volume (mm^3)		Change in volume (%)
	Day 0	Day 14	
Controls	660.1	1182.2	+79.1
Analog I (400 mg/kg p.o. 1× weekly)	500.3	288.2	−42.4

Table III. Comparison of the therapeutic ratio of retinoic acid and its analog I

Preparation	Hypervitaminosis A (min dose/day)	Papilloma (ED 50%/week)	Therapeutic Ratio
Retinoic acid	80 mg/kg	400 mg/kg	$\frac{400}{80} = 5$
Analog I	50 mg/kg	25 mg/kg	$\frac{25}{50} = 0.5$

The lower the ratio, the better the preparation.



I

Chemical structure of the retinoic acid analog I.

Skin papillomas and carcinomas. Skin papillomas and carcinomas were induced by means of 7,12-Dimethylbenz(a)anthracene (DMBA) and croton oil, as previously described in detail[2,3]. 150 µg DMBA were painted twice with an interval of 14 days on the back skin of Swiss albino mice followed by the applications of 0.5 mg croton oil twice weekly. Whereas skin papillomas appeared mostly 3–8 months after the first application of DMBA, carcinomas were usually not induced until after 5–12 months. Animals were chosen for the therapeutic tests, when they had developed multiple papillomas of an average diameter of 4 mm or a single carcinoma of at least 7 mm. The therapeutic experiments consisted in a 2 weeks' trial. Groups of 4 animals (with papillomas) or 11 (with carcinomas) were formed. They received once a week either intraperitoneally or orally a suspension of I in arachis oil. In the papilloma experiment, the average sum of the papilloma diameters per animal was determined for each group, on day 0 and day 14 of the trial. The increase or decrease of the papilloma diameters expressed in percent reflect the progression or regression of the papillomas. In the test with carcinomas, their approximate volume was determined by the product of length, width and height.

The controls showed a progression of papillomas by 23.2%. I has a marked effect on established papillomas. They regressed in a dose-dependent way under the treatment with I (Table I). After i.p. administration of 200 mg/kg I once a week, there was a reduction of the papilloma diameters of 74.1% within 14 days. In order to achieve a similar result with oral administration, it was necessary to double the dose to 400 mg/kg. Intraperitoneal application of doses of 50 mg/kg once a week still led to a decrease in the papilloma diameters by 55.4%. The effect on carcinomas was less pronounced. Whereas the mean carcinoma volume of the controls displayed a growth of 79.1%, the carcinoma volume of the animals treated with 400 mg/kg I once weekly orally, showed an average regression of 42.4% (Table II).

Hypervitaminosis A and therapeutic ratio. As already pointed out above, the limiting factor for therapy with retinoic acid is the so-called hypervitaminosis A syndrome, which in mice becomes clearly manifest in the form of weight loss, scaling of the skin, hair loss and bone fractures. In order to calculate a therapeutic ratio, we needed values for papilloma regression as well as for the induction of hypervitaminosis A, to be brought into relation with each other. To obtain a value for hypervitaminosis A a grading system of 0–4 – none to very marked – for each of the above-mentioned symptoms was used. It was specified which is the lowest daily i.p. dose causing in a 14 days' study (2×5 injections) a hypervitaminosis A. The latter was defined as being that condition of the animals when the addition of all the symptom grades yielded at least 3 (= hypervitaminosis A: min dose/day). The value for the anti-papilloma effect was assessed as the dose given i.p. once a week during a 2 weeks' therapeutic study – described above – leading to an approximately 50% regression of papilloma diameters (= papilloma: ED 50%/week). The therapeutic ratio =

$$\frac{\text{Papilloma: ED 50\%/week}}{\text{Hypervitaminosis A: min dose/day}}$$

enabled us to compare retinoic acid analogs with each other.

It can be seen from Table III that I possesses a 10 times more favourable therapeutic ratio than retinoic acid. Despite having this very marked activity against

chemically induced skin tumors, the aromatic analog I was similarly inactive as retinoic acid when tested against transplantable tumors like Ehrlich carcinoma, solid form, Ehrlich ascites tumor, Crocker sarcoma S 180 or leukemia L 1210 (BOLLAG[9]). Beside the direct antipapilloma effect, a metaplasia-preventing effect of I on the vaginal epithelium of the rat could be demonstrated in the modified colpokeratosis test of BOGUTH et al.[10]. With equimolar doses I showed 79% of the activity of retinoic acid (WEISER[11]). It would be very interesting to know whether the metaplasia or dysplasia preventing effect could be demonstrated also in other systems like organ cultures of tracheal epithelium[12] or prostate epithelium[13].

We may conclude that I, a retinoic acid analog, has a marked therapeutic effect on chemically induced benign and malignant epithelial tumors. With respect to the papilloma-regressing effect, I is superior to retinoic acid, as reflected in a 10 times better therapeutic ratio. This investigation has proved that the antitumor effect is not strictly linked with the development of hypervitaminosis A and that a dissociation between these two properties leads to a broader therapeutic margin. In preliminary studies it could be demonstrated that the aromatic analog I exerts also the same superiority over retinoic acid when given prophylactically. I delayed markedly the induction of premalignant as well as malignant epithelial skin lesions[9]. Clinical trials are undertaken on the therapy of precancerous conditions in man, with the goal to reach thereby a prophylaxis of malignant epithelial tumors.

Zusammenfassung. Retinsäure hat einen prophylaktischen und therapeutischen Effekt auf chemisch induzierte benigne und maligne epitheliale Tumoren. Die Wirkungen werden durch das Auftreten der sogenannten Hypervitaminose A-Symptome limitiert. Es werden die biologischen Eigenschaften eines aromatischen Retinsäure-Analogen (Figur) beschrieben, bei dem das Verhältnis zwischen den Dosen, die eine Tumorhemmung bewirken, und denen, die eine Hypervitaminose A erzeugen, 10mal günstiger ist als bei Retinsäure. Papillomregression und Hypervitaminose A sind nicht eng miteinander gekoppelt. Eine Dissoziation dieser biologischen Eigenschaften führt zu Substanzen mit besserem therapeutischem Quotient.

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On the Modification of the Divalent Cation-Binding by Phospholipids in Tumours

Phospholipids are the major structural components of the cell membrane and their combination with calcium and magnesium play a role of vital importance in many physiological phenomena, such as cell secretion[1], active transport[2], and cell permeability[3]. The isolation of phospholipid-calcium complexes from experimental tumours has been already reported[4].

A comparative study on the concentration of calcium and magnesium complexes with phospholipid in hepatoma, liver of hepatoma-bearing animals and liver from normal animals was carried out in the present experimental work.

8-day-old Novikoff hepatoma (ascitic or solid form) and 30-day-old hepatoma BW7756 bearing animals were used in this investigation. Groups of normal Holzman rats and C57L/J mice of the same sex and weight were used as control groups. In order to isolate the phospho-

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Calcium and magnesium complexed by the phospholipids of neoplastic and normal tissues

	Total lipid phosphorus (μg P/g tissue)	Complexed phospholipid (% of total lipid P)	Complexed calcium (μg Ca/g tissue)	Complexed magnesium (μg Mg/g tissue)
Novikoff hepatoma[b]				
Solid form	114 (91–156)[a]	9.2 (5.8–14.0)[a]	3.9 (2.2–6.3)[a]	1.1 (0.7–1.6)[a]
Ascites form	201 (190–223)	8.3 (6.5–11.0)	1.7 (1.0–3.1)	2.9 (1.8–4.2)
Liver solid tumor bearing	340 (232–424)	6.3 (2.7–12.9)	3.5 (2.7–4.2)	5.8 (2.8–7.3)
Liver ascites bearing	340 (241–451)	13.0 (10.8–16.6)	4.5 (4.1–5.2)	8.1 (7.2–9.3)
Liver normal animals	436 (386–500)	12.7 (6.4–18.8)	4.2 (2.8–5.5)	10.1 (7.9–11.4)
Hepatoma BW7756[c]				
Solid tumor	23 (15–28)	7.4 (5.2–9.0)	2.5 (1.9–3.0)	1.5 (1.2–1.6)
Liver tumor bearing	66 (51–71)	9.6 (7.2–11.1)	3.3 (3.0–3.9)	4.8 (4.3–5.7)
Liver normal animals	95 (93–96)	7.3 (6.4–8.9)	2.1 (1.9–2.4)	3.6 (3.4–3.8)

[a], Range; [b], from 20 animals; [c], from 45 animals.