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.

lipid complexes, the tumour and liver were processed as described elsewhere[4]. After mineralization of the fractions obtained with $HClO_4$ plus HNO_3, phosphorus, calcium and magnesium were measured in aliquots of the mineralized material. Phosphorus was assayed colorimetrically using aminonaphtolsulfonic acid reagent[5]. Calcium and magnesium were determined with an Atomic Absorption Spectrometer. The results shown in the Table are the average and the range values corresponding to each type of tumour.

The observation that tumor tissue contains a much lower content of phospholipid than liver is in agreement with that of other authors[6,7]. The most striking difference between hepatoma and normal liver is a much lower amount of magnesium complexed by the phospholipids, while the complexed calcium does not show significant variations. Since it has been demonstrated that the binding of calcium and magnesium by the membranes exhibits a saturation type relationship characteristic of adsorption to binding sites and describable by the law of mass action[8], this difference may be due to a considerably higher calcium concentration at the cell membrane level. On the other hand, considering that tumor has shown a slightly higher proportion of cephalin than liver[9], and that in biphasic systems the acidic phospholipids have shown a far greater affinity for Ca^{2+} than for Mg^{2+}[10], it is possible that this small difference in the phospholipid composition may also contribute to the magnesium-binding behavior observed.

These findings raise the question whether this change in the divalent cation distribution may or may not be implicated in determining the tumor cell membrane characteristics. In relation to this, it is also very interesting to note that, with less than half of the complexed phospholipid amount, tumors bind almost as much calcium as liver does. This is an indication of a drastic change in the phospholipid-calcium relationship.

Zusammenfassung. Phospholipid-Kalzium- und Phospholipid-Magnesium-Komplexe wurden aus Hepatom und aus normaler Leber isoliert. Obwohl das Hepatom nur die Hälfte der in der Leber vorkommenden komplexen Phospholipide aufweist, ist die Kalziumbindung bei Hepatom und Leber gleich. Die Magnesiumbindung ist dagegen erheblich niedriger. Diese Unterschiede weisen auf eine mögliche Bedeutung der zweiwertigen Kationenbindung im Verhalten der Tumorzellmembran hin.

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On the Quarternary Structure of *Carcinus moenas* (Arthropoda) Hemocyanin[1,2]

It is well enough established that the so-called minimum functional subunit of the hemocyanin (Hc) of several Arthropoda species (unlike Mollusca[3]) can be isolated without covalent bond cleavage[4,5]. This component includes one active site with 2 copper ions, weighs around 75,000 d and has been reported to be rather heterogeneous[6-9]. Three different hypotheses about its constitution have been proposed following the studies on minimum subunits regardless of their functionality: 1. Pickett, Riggs and Larimer[10] have reported that succinylated *Homarus americanus* Hc displays by sedimentation analysis a subunit of about 37,500 d, which probably consists of only one polypeptide chain; 2. according to another model[11], suggested in order to explain the low and 'continuously varying' sedimentation coefficient of *Cancer magister* Hc in 6 *M* guanidine hydrochloride solution, the minimum functional subunit is composed of 3 polypeptide chains of 25,000 d. Such a model was made probable by recent data obtianed in our laboratory on *Carcinus moenas* Hc by polyacrylamide gel electrophoresis and gel permeation chromatography in 0.1% SDS solutions at pH 7.0–9.3[12,13]; 3. the results of Loehr and Mason[8], confirmed by Carpenter and van Holde[9], seem to prove that the subunits around 80,000 d of *Cancer magister* Hc consist of single polypeptide chains. These results have been obtianed by polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS) solutions at pH 8.0–9.0.

We have separated *Carcinus moenas* Hc polypeptide chains using gel permeation chromatography on Sephadex G-200 in 1% SDS solution[14,15]. Hc and apoHc (apohemocyanin) were prepared according to Ghiretti

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