driven to a 'lethal proliferation'. The growth becomes unbalanced to such an extent that the cells do not survive.

A stimulation of the defence mechanism may also play a role in the regression of skin papillomas. Spontaneous regression of papillomas occur. It was shown that X-rays and thymectomy delayed, whereas a methanol extract of bacille Calmette-Guérin (BCG) accelerated the regression of papillomas (LAPPÉ and PREHN[22]). It was assumed that an immunologic surveillance is operative with regard to premalignant skin papillomas. The balance can be disturbed in both ways. Whereas immunosuppressive measures like irradiation and thymectomy may decrease the regression rate by diminishing immune reactions, immunostimulating agents, like extracts of BCG acting as adjuvants may enhance the regression rate. Vitamin A has been shown to have an adjuvant effect. Under the influence of retinol it was possible to obtain a certain titer of humoral antibodies towards an otherwise non immunogenic protein (DRESSER[23]).

The above mentioned results show that it is possible to induce regressions of a benign epithelial tumor by compounds which do not belong to the well known classes of cytotoxic or antimitotic agents. Vitamin A compounds do not suppress mitotic activity but even enhance it. Thus the mode of action must be very different from that of the compounds used until now in the chemotherapy of tumors. After these first positive results in the treatment of a benign epithelial tumor we tried in animal as well as in clinical experiments to influence malignant epithelial tumors. We succeeded to induce partial and in a few cases even total regressions of chemically induced skin carcinomas of mice arising from papillomas (BOLLAG[11]). Furthermore, in clinical trials locally applied retinoic acid besides reducing or eliminating actinic or senile keratoses, considered as precancerous epithelial lesions also caused partial or complete regressions of basal cell carcinomas of the skin (BOLLAG und OTT[24]). Thus vitamin A and particularly vitamin A acid may be considered as therapeutic agents affecting special types of tumors, but possessing a mode of action completely different from that of the known cancer chemotherapeutic agents.

Zusammenfassung. Mittels Dimethylbenzanthracen und Krotonöl wurden bei Mäusen Hautpapillome erzeugt. Diese Tumoren wurden durch systemische Anwendung von Retinylpalmitat und Retinsäure therapeutisch beeinflusst. Es kam zu einer deutlichen Regression der Tumoren, die dosisabhängig war. Sowohl orale wie parenterale tägliche oder wöchentliche Applikation der geprüften Vitamin-A-Verbindungen waren wirksam. Der Wirkungsmechanismus wird diskutiert. Er unterscheidet sich grundsätzlich von demjenigen anderer tumorhemmender Substanzen.

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Interaction of Lead with Erythrocytes

Blood lead is found mainly in association with the erythrocytes and only 5% or less is in the plasma. Studies with radioactive lead (Pb-203) in vitro have shown that plasma is rapidly cleared of lead by erythrocytes and that equilibrium is reached after approximately 15 min (unpublished data). The kinetics of the reaction in vivo using Pb-210 have been reported by other authors[1] with essentially similar findings. The nature of this interaction has been the subject of speculation. Most workers have assumed that the principal site involved was the erythrocyte membrane. AUB et al.[2] postulated that lead was precipitated at the surface of the membrane as the phosphate, but later workers suggested that it was as the diphosphoglycerate[3]. Support for this view was given by the demonstration that membrane permeability was altered by low concentrations of lead[4,5]. The interference with ATP synthesis within the cell has also been attributed to the interaction of lead with a ligand at the cell membrane rather than with an intracellular ligand[6]. Conversely, lead is known to traverse the plasma membrane of nucleated cells without difficulty[7,8] and also that it traverses the erythroblast membrane since mitochondrial abnormalities result in that cell series in experimental lead poisoning[9]. Evidence also exists for a lead binding compound within the erythrocyte[10,11].

We have therefore re-examined the lead-binding properties of human erythrocytes and attempted to identify the cell fractions in which binding occurs. 2 techniques have been employed to separate erythrocytes into fractions of varying molecular weight, namely, Sephadex gel filtration and ultracentrifugation. Fresh, washed erythrocytes were haemolyzed by freezing and thawing, and diluted 10-fold with *tris*-maleic acid buffer pH 7.0. 5 ml of haemolysate was passed upwards through a 100 × 2.5 cm Sephadex 200 column at 20 ml/h which had been calibrated with cytochrome C (Sigma Chemical Co., St. Louis); transferrin (Sigma Chemical Co., St. Louis) and γ-globulin (A. B. Kabi, Stockholm). The elution volumes were cytochrome C 395 ml, transferrin 325 ml, γ-globulin 290 ml. The effluent was monitored with a double-beam, flow-through UV-analyser at 254 nm (ISCO, model UA) prior to collection in 10 ml aliquots with a drop counting

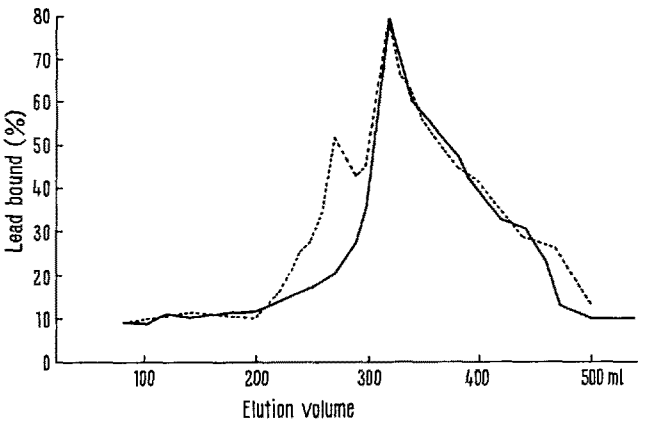

Fig. 1. Gel filtration of human erythrocyte haemolysates on a column of Sephadex G-200. Elution with *tris*-maleic acid buffer pH 7.0. Lead binding of fractions determined at equilibrium by ultra-filtration ....., before; ——, after preliminary ultra-centrifugation at 60,000*g* for 4 h (3 × 23 ml M.S.E. 65 swing-out rotor).

fraction collector (L.K.B. Ultrorac 7000). Each aliquot was subjected to protein[12] and haemoglobin[13] determination. An aliquot of each fraction was introduced into a dialysis tubing bag (Visking) previously soaked for 24 h in several changes of distilled water. Solutions of lead chloride labelled with radioactive lead (Pb-203) were added to a final concentration of 10 ppm. After sealing, the bags were supported on large-pore, sintered-glass, long-stemmed filter crucibles in centrifuge tubes and spun for 2 h at 2500g in a refrigerated centrifuge. The γ-activity in aliquots of this solution and of the ultrafiltrate was determined (Hewlett-Packard Auto-Gamma Spectrometer) and the percentage of lead bound obtained from the difference in count rates (Figure 1). The haemoglobin content of each fraction was determined before and after centrifugation (Figure 2) and compared with the lead binding characteristics. The protein content of each aliquot closely resembled that of haemoglobin.

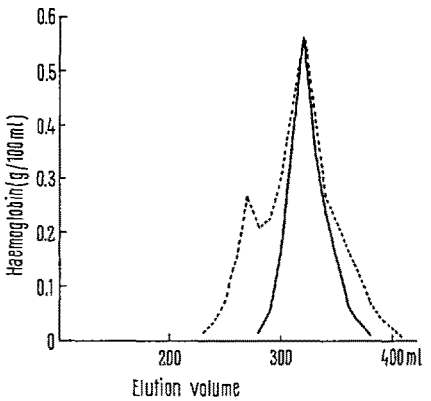

Fig. 2. Haemoglobin concentrations in fractions obtained by gel filtration (Sephadex G-200) and human erythrocyte haemolysate. ....., before; ——, after preliminary ultra-centrifugation at 60,000g for 4 h (3 × 23 ml M.S.E. 65 swing-out rotor).

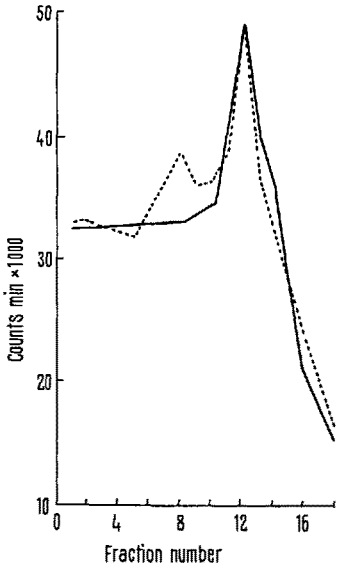

Fig. 3. Density – gradient ultra-centrifugation of human erythrocyte haemolysate. The sucrose gradient (1.9–1.2M) contained a uniform concentration of lead (10 ppm) labelled with Pb-203 and was spun in a 3 × 23 ml M.S.E. 65 swing-out rotor for 16 h at 50,000g. Binding is indicated by the increased activity above the initial plateau levels and diminished activity in the fractions through which the binding material has passed. ....., before; ——, after preliminary ultra-centrifugation at 60,000g in the same rotor.

Samples of haemolyzed blood without added lead were also layered onto a sucrose gradient (1.2–1.9M) which contained a uniform lead concentration (10 ppm) and labelled with Pb-203. This was spun for 16 h at 50,000g (M.S.E. 65 3 × 23 ml swing-out rotor) to separate haemoglobin from the stromal layer. After fractionation of the gradient, measurement of the radio-activity allowed the distribution of lead to be determined (Figure 3). Lead binding was demonstrated by increased activity in the haemolysate fractions and decreased activity in the upper part of the gradient which they had traversed. Both procedures were repeated after preliminary centrifugation of the haemolysate at 60,000g for 4 h and this resulted in the disappearance of the pre-haemoglobin zone with lead binding activity.

The results suggest that lead is bound to the cell contents rather than to stromal material. This was confirmed by the absence of significant binding of lead when washed stroma replaced haemolyzed erythrocytes. The interaction with the prehaemoglobin band can be attributed to residual haemoglobin bound to that fraction, comparison of the elution characteristics of this band with the calibration graph indicated a mean molecular weight of 240,000. Stromal proteins extracted from red cell ghosts have been shown to have molecular weights ranging from 10,000 to 170,000[14], however these values are unlikely to apply to material obtained by simple haemolysis. The lack of correspondence between haemoglobin concentration and lead binding in fractions obtained from the Sephadex column at elution volumes of 350 ml and above suggests that there may be some low molecular weight material in addition to haemoglobin that interacted with lead[15].

Zusammenfassung. Untersuchungen mit ^{203}Pb in vitro ergeben, dass Blei in den Erythrozyten vorwiegend an Hämoglobin gebunden wird, während Stromasubstanzen kein Blei aufnehmen.

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