present in the alveolar luminae, than were the glands of animals kept in continuous darkness. The alveolar cells of glands from animals kept in continuous light were cylindrical, fairly uniform in appearance, stuffed with small vacuoles (Figure 1). This type of alveolar cell could also be seen in glands from animals kept in continuous darkness (Figure 2). In addition these animals showed another type of cell with very large vacuoles (Figure 2).

The uterus from hamsters kept in darkness for 72 days atrophied whereas continuous light for 72 days resulted in a much thicker uterus.

Discussion. After 72 days of continuous light the porphyrin concentration and content in the Harderian gland was increased more than 4-fold compared with animals kept in darkness, despite a higher mean gland weight for the latter. This could be a direct effect of light on the Harderian gland or an effect via the eye and the pineal gland[1], which in its turn could be direct or mediated through the hypothalamic-adrenal-gonadal axis[2]. The high content of porphyrin in glands from animals kept in continuous light was parallelled histologically by a higher amount of luminar pigment in these glands than in animals kept in darkness. The large-vacuolated alveolar cells seen in the latter animals could sometimes be seen in animals kept on the diurnal lighting condition 12 L: 12 D but never in animals kept in continuous light. There may thus be 2 basic types of alveolar cells of which only 1 seems to respond to changes in lighting conditions. 2 types of secretory cells are previously described in the rat[5] and the mouse[6].

The morphology of the hamster uterine tissue showed hypertrophy after continuous light and atrophy in animals kept in darkness. The same characteristic morphological responses of the uterus to different lighting regimes have previously been described by REITER et al.[7].

Zusammenfassung. Bei Goldhamstern, welche längere Zeit im Dunkelversuch (72 Tage) gehalten wurden, konnten in der Harder'schen Drüse zwei Zellarten nachgewiesen werden, bei konstantem Licht dagegen nur eine einzige Zellart. Der Porphyringehalt in diesen Drüsen ist bedeutend höher.

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Pea Phytohemagglutinin Selective Agglutination of Tumour Cells

The recently characterized phytohemagglutin of pea[1], the biological activity of which has not yet been established, was compared with concanavalin A (con A) for the ability to agglutinate tumour cells. Pea phytohemagglutinin was prepared as a mixture of 2 substances of the same molecular weight, of very similar amino acid composition, and identical hemagglutinating activity[1]. Con A lyophilized in NaCl was purchased from Calbiochem (grade A, lot 010229). For agglutination assay cells all cultivated in vitro were harvested with 0.02% EDTA solution in PBS and washed 3 times with PBS. Normal embryonic fibroblasts of human (HuEF), rat (LWF) and RIF-free chicken (BLEF) origin were examined. Tumour cells were represented by one clonal line of spontaneously in vitro transformed Lewis rat fibroblasts (LW13K2)[2], 3 Rous sarcoma virus in vitro transformants of LW13 cells (LW13-RsK4[3], RsK4-A4[4], RsK4-A4K1[4]) and 1 in vivo induced Rous Wistar rat sarcoma (CZW1)[4]. The agglutination assay was done in test tubes. To 0.2 ml of phytohemagglutinin solution 0.2 ml of cell suspension was added. The results were read after 15 min both macroscopically and microscopically. Trypsinization (Trypsin Spofa, Czechoslovakia, crystallized) of normal cells was carried out in suspension at trypsin concentration of 0.1% in PBS for 6 or 10 min at 37 °C. Trypsin action was stopped by soybean trypsin inhibitor. 30 m*M* methyl α-D-glucopyranoside was used to inhibit phytohemagglutinins in a concentration of 500 μg/ml. The mixture of phytohemagglutinin and hapten was incubated at least 15–30 min before the addition of cell suspension.

Agglutinability of normal and tumour cells by con A and pea phytohemagglutinin is shown in the Table. Normal rat and human cells show the usual patterns of interaction with phytohemagglutinins. They are agglutinated only when the highest concentration (1500 μg/ml) of both con A and pea phytohemagglutinin is used. After trypsin treatment the amount of phytohemagglutinins necessary for minimum significant agglutination of normal cells decreases – less for con A and more for pea phytohemagglutinin. Agglutinability of in vitro spontaneously transformed highly malignant rat tumour cells LW13K2 lies between normal cells and rat Rous sarcoma cells. Rat Rous sarcoma cells are agglutinated even at a concentration of 10 μg of con A per 1 ml or 5 μg of pea phytohemagglutinin per 1 ml. Clumping of cells caused by pea phytohemagglutinin is always easier to read than a similar effect of con A as pea phytohemagglutinin leaves less free single cells in suspension. Specificity of phytohemagglutinin tumour cell agglutination is in all positive cases proved by the inhibition of agglutination by sugar. In our experiments methyl α-D-glucopyranoside was chosen for its relatively high inhibitory activity for both pea phytohemagglutinin and con A[5]. High degree of inhibition of tumour cell agglutination is obtained only when the sugar is preincubated with phytohemagglutinin solutions for 15–30 min before the addition of cells. Addition of sugar to already agglutinated cells had no

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Agglutination of cells by concanavalin A and pea phytohemagglutinin

Cells	0.1% trypsin treatment (min)	Concanavalin A concentration (μg/ml) 1500	1000	750	500	200	100	50	25	10	5	1	500 μg/ml in 30 m*M* sugar
LWF		−	−	−	−	−	−	−	−	−	−	−	
	6	+	+	+	+	−	−	−	−	−	−	−	−
HuEF		±	−	−	−	−	−	−	−	−	−	−	
	10	+	+	+	+	+	±	−	−	−	−	−	−
BLEF		+	−	−	−	−	−	−	−	−	−	−	
	10	±	−	−	−	−	−	−	−	−	−	−	−
LW13K2		+	+	+	+	+	±	−	−	−	−	−	−
[a])		+	++	++	+	+	±	−	−	−	−	−	−
LW13-RsK4		+	+	+	+	+	+	±	−	−	−	−	−
RsK4-A4		+	++	++	++	++	++	++	+	+	−	−	−
RsK4-A4Kl		+	+	+	+	+	+	+	+	−	−	−	−
CZW 1		+	+	+	+	+	+	−	−	−	−	−	−
		Pea phytohemagglutinin concentration (μg/ml)											
LWF		+	−	−	−	−	−	−	−	−	−	−	
	6	++	+	+	+	+	+	+	+	±	−	−	−
HuEF		+	+	±	−	−	−	−	−	−	−	−	
	10	++	+	+	+	+	±	−	−	−	−	−	−
BLEF		+	±	−	−	−	−	−	−	−	−	−	
	10	+	+	+	+	−	−	−	−	−	−	−	−
LW13K2		++	++	++	+	+	+	±	−	−	−	−	−
LW13-RsK4		++	++	++	+	+	+	+	±	−	−	−	−
RsK4-A4		+++	+++	+++	+++	++	++	++	+	+	−	−	−
RsK4-A4Kl		+++	+++	+++	++	++	++	++	+	+	+	−	−
CZW 1		++	++	++	++	+	+	+	±	−	−	−	−

The cell concentrations used in agglutination assay ranged from 0.8×10^6 to 2.5×10^6 cells/ml. The appropriate range of concentrations was determined by pretesting to meet rigid serological criteria for reading agglutination. One plus sign (+) designates only small macroscopically observable clumps resisting a gentle shaking with test tube; 2 plusses (++) = clumps at least twice big as one plus clumps; 3 plusses (+++) = very large clumps only. The plus-minus sign covers a range of microscopical clumps (3-20 cells) mixed with numerous free single cells. [a]) In this case con A prepared by the Agrawal and Goldstein procedure was tested for comparison with the all other results obtained with con A manufactured by Calbiochem.

'uncoupling' effect. The slightly higher and more pronounced agglutination activity of pea phytohemagglutinin is probably due to its higher molecular uniformity at physiological pH[1].

Zusammenfassung. Phytohaemagglutinin, aus der Erbse *Pisum sativum* L. isoliert, agglutiniert vornehmlich Tumorzellen, ähnlich wie Concanavalin A. Die durch RNS-onkogene Viren transformierten sowie spontan transformierten Zellen befolgen während der Agglutination das normale Muster der Tumorzellen-Interaktion mit Concanavalin A oder Weizenkeim-Agglutinin.

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Phospholipid-Calcium Complexes in Experimental Tumors

Experimental tumors show a very high calcium uptake[1]. A large amount of the incorporated ^{45}Ca is localized in the microsomal fraction[2] which contains fragments of many cellular membranes[3]. It is possible that besides the contribution of the probable nucleic acid-calcium interaction[2] a very significant part of this incorporation is due to phospholipid-calcium complex formation. A recently reported increase in tumors of the membrane-bound calcium[4] appears to corroborate this assumption. Furthermore, it has been demonstrated that the binding of calcium by acidic phospholipids, phosphatidylserine (PS) and phosphatidylethanolamine (PE), is enhanced in the presence of phosphate ion[5].

In the present experimental works the phospholipids from Ehrlich ascites, lymphatic leukemia BW5147 and lymphosarcoma 6C3HED were extracted using the organic phase of a mixture of chloroform-methanol-water (2:1:2 by volume). The extract was washed 3 times with