Effects of β-palmitoyl-lysolecithin on isolated guinea-pig ventricles.

Concentration of lysolecithin, μg/ml	Onset of negative inotropic effect after	Onset of irregularities after	Onset of negative chronotropic effects after	Remarks
80	7 min	none	none	Washed with NKR after 10 min no reversal of effects
105	1.5 min	none	1.5 min	Washed with NKR after 3 min no reversal of effects
105	2.5 min	2.5 min	1.7 min	
200	2 min	2.5 min	1.2 min	Slight (20 %) positive inotropic effect in 1.5 min lasting for 0,5 min

The isolated guinea-pig ventricles were prepared as before[6], except that contractile force was recorded through a force-displacement transducer on a Grass polygraph. Records were made while the ventricle was perfused with normal Krebs-Ringer solution (NKR), then with the same solution in which half of the Ca was replaced with Na. Lysolecithin was added to the low-Ca solution, so that inotropic effects could be observed in the hypodynamic heart.

Results. (A) Erythrocytes. Four experiments were made with concentrations of lysolecithin from 30 to 200 μg/ml. In no case did the compound affect the rate of loss of K^{42} from the plasma. The highest concentration was about 4×10^{-4} *M*. This is 10^4 times the concentration of ouabain which inhibits K transfer by 50 %, and 400 times that of digitoxin[5]. Hence it seems unlikely that palmitoyl lysolecithin shares with the cardiac glycosides the ability to inhibit active K transport in human erythrocytes. Haemolysis was not greater in blood containing lysolecithin than in control samples. On the other hand, dilute suspensions of erythrocytes in buffered saline (0.125 % red cells) were haemolyzed by lysolecithin in concentrations as low as 10 μg/ml.

(B) Guinea-pig ventricles. Four experiments were made with this preparation which are summarized in the Table. In all four hearts the phosphatide had negative inotropic effects which progressed to the point of diastolic arrest. Contracture (rise of the base line) was never seen. All of these effects are different from those of cardiac glycosides which have been described before[7]. Negative chronotropic effects were seen with doses above 80 μg/ml. The irregularity, seen in one experiment with 105 μg/ml and in the one with 200 μg/ml, was a series of ventricular extrasystoles. A typical sequence of events is illustrated in the Figure. In three of the four experiments with lysolecithin, perfusion with NKR was resumed after the effects of the phosphatide were seen. In every case, the effects of the drug were unaffected by washing, and progressed to the termination of the experiment. In the experiment with the highest concentration of phosphatide, there was a slight transient positive inotropic effect (see Figure) which was quickly replaced by a more severe and longlasting negative inotropic effect. This was the only case in which there was any evidence of increased contractile force.

Effects of β-palmitoyl lysolecithin (200 μg/ml) on an isolated guinea-pig ventricle.
[1] NKR control. [2] Low-Ca control. [3] 1.8 min after lysolecithin. Note slight positive inotropic effect (not observed in the other experiments). [4] 3 min after lysolecithin. Irregularities have begun; contraction height decreasing. [5] 8 min. [6] 14 min. Severe effects, which progressed in 2 min to diastolic arrest. [7] 16 min. Diastolic arrest.

Our results do not agree with those of HAJDU et al. In view of the differences in the test systems used and of the different starting materials and procedures applied for the preparation of the lysolecithins, it is difficult to interprete the discrepancies. We feel, however, that our data present conclusive evidence that pure β-palmitoyllysolecithin, as prepared according to HANAHAN et al., does not possess any activity similar to that of the cardiac glycosides on the human erythrocyte and the isolated guinea-pig ventricle[8].

Zusammenfassung. 1957 sind von HAJDU et al. Versuche veröffentlicht worden, wonach sich aus verschiedenen Geweben eine Substanz mit Herzglykosid-ähnlicher Wirkung auf Kaltblüterherzen extrahieren lässt, welche als β-Palmitoyl-lysolecithin interpretiert wurde[1]. In unseren Versuchen wurde aus Hefe Dipalmitoleyl-lecithin isoliert und daraus durch enzymatische Abspaltung der α-ständigen Fettsäure und anschliessende katalytische Hydrierung reines, hämolytisch wirksames β-Palmitoyl-lysolecithin hergestellt. Diese Verbindung zeigte jedoch keine den Effekten der Herzglykoside verwandte Wirkung am isolierten Meerschweinchenventrikel sowie an menschlichen Erythrocyten.

J. B. KAHN, jr. and R. SCHINDLER

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Occurrence of Virus-Like Particles in Cultured Cloudman S-91 Melanoma

In connection with recent cytological studies of cultured cells of the Cloudman S-91 mouse melanoma[1], further electron microscopic observations have revealed the presence of virus-like bodies in tumor cells cultivated *in vitro*. The present report concerns itself with a description of these virus-like particles, indistinguishable struc-

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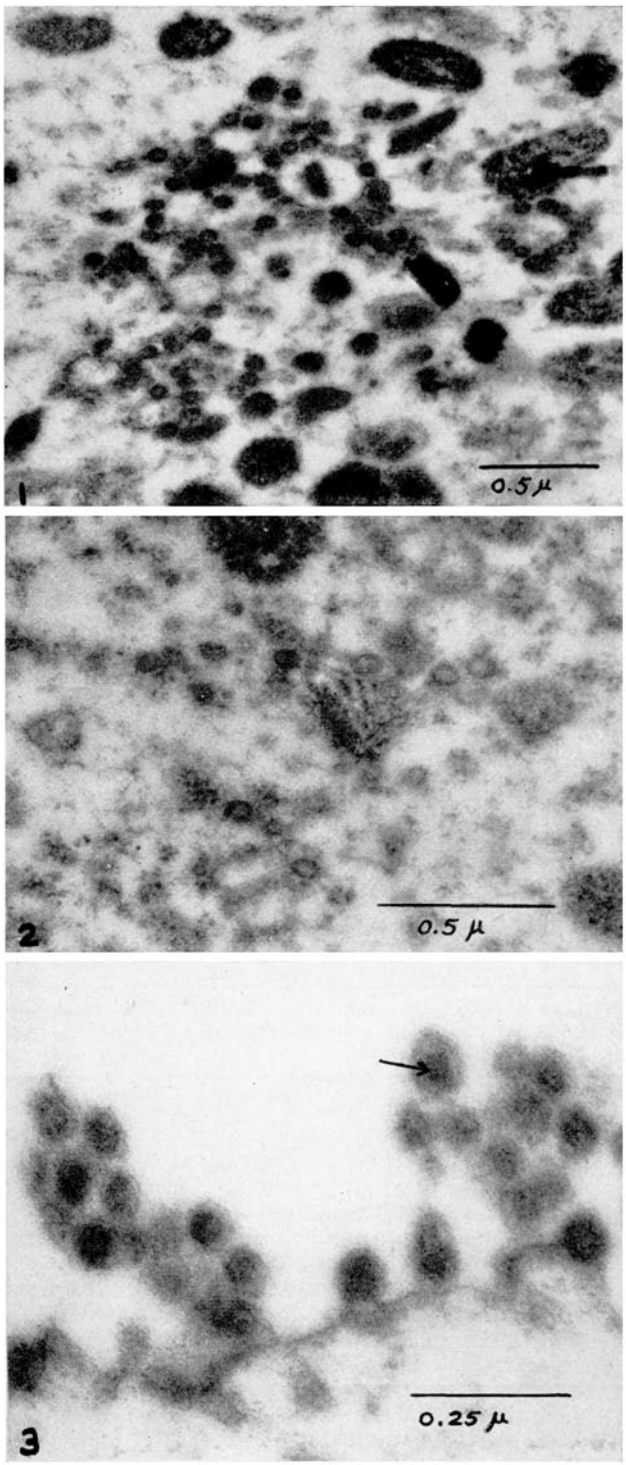
turally from particles found in mouse mammary tumors[2], and with their possible relation to virus-like bodies observed in a number of mouse neoplasms.

Mice of the dba/2 strain, bearing subcutaneous transplants of the Cloudman S-91 mouse melanoma, were obtained from the Cancer Research Genetics Laboratory of the University of California in Berkeley. Following sacrifice by cervical fracture, the melanoma tissue was removed under sterile conditions and fragments explanted in chicken plasma clots in milk dilution bottles. The nutrient medium consisted of 5% chick embryo extract, 20% lamb serum and 75% Hanks balanced salt solution. The medium was changed on the seventh day. On the tenth day the fluid was poured off and the tissue and outgrowth fixed in osmium tetroxide vapor for 10 min, followed by 30 min fixation with buffered sucrose-osmium tetroxide solution[3]. Following dehydration, fragments of outgrowth were embedded in methacrylate, ultra-thin sections mounted on carbon-coated copper specimen grids, and stained with uranyl acetate[4]. All sections were examined with an RCA EMU-2B electron microscope.



Figures 1–3. Virus-like particles in Cloudman S-91 melanoma cells in culture. See text for description.

The particles commonly observed are of two distinct types. Occurring predominantly in the cytoplasm (Figure 1, 2) is the ring-shaped profile, appearing as a somewhat electron dense vesicle delimited by two strongly osmiophilic membranes and with an outer diameter ranging from 60 to 80 mμ. The particles are seen scattered through the cytoplasm, gathered into irregular elleptical or circular clusters, and surrounding membrane-bound intracytoplasmic vacuoles. In Figure 3 is depicted a larger extracellular form, 70 to 110 mμ in diameter, with a concentric double membrane enclosing in some instances an eccentric dense nucleoid (arrow), sometimes observed to be delimited by a third, closely-applied membrane. Also seen is the extrusion of virus-like particles through protrusions of the cell membrane. It should be noted that whereas few viral bodies could ever be observed in ultra-thin sections of fresh melanoma tissue, they were readily apparent in sections of cultures of the neoplastic tissue, an observation similar to Lasfargues' experience with mouse mammary carcinoma[5]. The intra- and extracellular particles observed here are morphologically comparable to the virus-like particles described in mammary tumors[6–8]. Along these lines, Fawcett and Wilson[9] observed virus-like particles in spontaneous hepatomas of C3H male mice and inferred their similarity to virus-like particles seen in mammary tumors. Howatson and McCulloch[10] described virus-like particles indistinguishable from the intracytoplasmic forms observed in mammary tumors in C3H mice in a mouse plasma cell tumor originating in a $22^1/_2$ month old female C3H/He mouse in the Cancer Research Genetics Laboratory in Berkeley and serially transplanted in C3H/He mice. The first instance of the occurrence of these characteristic particles in a chemically (3, 4-benzopyrene) induced carcinoma of the uterine cervix was reported by Thiery et al.[11] in virgin C3H/A mice, which are known to have the milk factor and to be disposed to mammary carcinoma. Comparable but not identical structures have also been observed in intracytoplasmic vacuoles of Cloudman S-91

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melanoma[12], Ehrlich ascites mouse tumors[13], and in FRIEND's mouse leukemic material[14].

The similarity of the virus-like particles revealed in various neoplasms of mice is reminiscent of earlier observations of viral particles in chicken neoplasms[15]. It might be hypothesized that these virus-like bodies are present as an inactive ubiquitous agent in mice and are acquired fortuitously as a non-specific contaminant in spontaneous or experimentally induced tumors, or vicariously during numerous serial tumor transplantations. THIERY et al.[11] have suggested that these structures may be a result rather than the cause of the neoplastic reaction. It might perhaps be postulated that these particles are identical, the nature and localization of the neoplastic process being determined in part by host responses. Alternatively, these bodies may represent distinct entities, indistinguishable by electron microscopy, and each producing specific neoplasms. Substantiation of any of the proposed hypotheses will require more extensive investigation into a variety of mouse tumors.

Zusammenfassung. Elektronenmikroskopische Untersuchungen des Cloudman-S-91-Melanomagewebes ergaben das Vorhandensein virusähnlicher Teile in der Kultur, die sich strukturell nicht unterscheiden lassen von den in Mäuse-Mammatumoren beobachteten. Die inneren und äusseren Gewebsteilchen waren morphologisch mit verschiedenen Mäuseneoplasmen vergleichbar.

B. V. SIEGEL and S. R. WELLINGS[16]

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Hypervitaminosis in an Insect Larva

It is believed that B-vitamins in high concentrations may have adverse effects on the metabolism of an organism[1]. Not much is known about the effect of administering higher concentrations of B-vitamins in the case of insects. The observations on the effect of feeding the larva of rice moth, *Corcyra cephalonica* (Stainton) with higher doses of biotin, nicotinic acid and riboflavin are presented here.

Corcyra larva has been successfully grown on a basal diet consisting of casein, dextrose, cholesterol, McCollum-Davis salt mixture No. 185, a mixture of B-vitamins and linoleic acid[2]. It has been shown that thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, choline chloride, *p*-aminobenzoic acid, folic acid and biotin are essential for the normal growth of the larva, the latter, however, being needed only when linoleic acid is withheld from the diet[2]. The diet used in the experiment on the administration of graded doses of biotin did not, therefore, contain linoleic acid. Table I shows the results of the test on the effect of graded doses of biotin on the growth of the larva. It is apparent that the higher concentrations of this vitamin have adverse effects on growth as indicated by decrease in weight.

The response of the larva to the administration of nicotinic acid (Table II) beyond a level of 100 µg/g of diet is suggestive of some disturbance in growth, though this

Tab. I. Growth of *Corcyra* larva on diets containing graded doses of biotin

Amount of biotin (in µg/g)	Average weight (in mg) of larva on 12th day	19th day	26th day	Percentage of survival
0.00	3.3	7.8	16.4	40.0
0.25	6.1	23.7	32.6	46.6
0.50	5.3	22.8	30.8	40.0
1.0	5.7	24.5	34.1	46.6
2.0	4.8	14.5	29.6	50.0
5.0	5.3	16.3	22.2	40.0
10.0	5.6	20.4	24.4	30.0
25.0	5.0	16.4	23.2	30.0
50.0	5.8	17.8	22.9	40.0

Tab. II. Growth of *Corcyra* larva on diets containing graded doses of nicotinic acid

Amount of nicotinic acid (in µg/g)	Average weight (in mg) of larva on 12th day	19th day	28th day	Percentage of survival
0.0	0.5	1.0	1.0	6.6
2.5	5.6	11.6	16.4	40.0
5.0	6.4	25.3	34.3	46.6
10.0	6.6	28.4	34.1	56.6
15.0	6.1	25.5	pupated	46.6
25.0	7.7	23.8	pupated	53.3
30.0	6.8	29.0	pupated	50.0
50.0	6.2	28.5	33.0	56.6
75.0	6.1	30.1	40.2	50.0
100.0	2.9	24.4	41.1	46.6
150.0	3.4	23.3	40.0	53.3
200.0	2.9	21.0	42.5	60.0

Tab. III. Growth of *Corcyra* larva on diets containing graded doses of riboflavin

Amount of riboflavin (in µg/g)	Average weight (in mg) of larva on 12th day	20th day	29th day	Percentage of survival
0.0	5.8	15.1	34.8	53.3
2.5	6.0	16.6	pupated	53.3
5.0	5.9	18.2	36.9	50.0
10.0	6.1	15.4	32.5	56.6
15.0	6.9	17.5	36.2	50.0
20.0	6.0	16.6	34.2	46.6
25.0	6.4	18.8	32.7	50.0
30.0	6.5	18.0	34.8	50.0
40.0	5.9	17.2	30.4	46.6
50.0	4.8	16.6	pupated	43.3
75.0	3.7	14.5	17.2	40.0
100.0	3.2	15.3	18.0	43.3

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