at the same force, for another 60 min (Table I, groups 6 and 7). The incidence of the tumour in both groups is 100% but an important difference of 64 days is observed in its mean appearance.

In the centrifugation experiment of the tumour extract over 2 layers of sucrose (group 8), the injection of the 5 different fractions collected from top to bottom leads to tumour development. Results as well as mean interval between injection and observation of the tumour are given in Table II. In this experiment again a slight sedimentation effect is noted. Nevertheless, it seems that a centrifugation of the tumour extract at 130,000 × *g* for 45 min, followed by further centrifugation of the resulting supernatant at 150,000 × *g* for 120 min is not sufficient to sediment completely the tumour-inducing agent. These results are not consistent with those of other workers who found that the *Xenopus laevis* 'lymphoid tumour' agent is gradually lost from the supernatant fluid during centrifugation at 40,000 × *g*[8].

In the filtration experiments the incidence of the tumour is reduced. 76% and 58.8% of the young *Xenopus laevis* which received the filtrate after passage through 450 nm filter, and in 53% of those injected with filtrate obtained by using the 220 nm filter, were cancerous. The adult animals develope the tumour in 33% of the cases (compare Tables I and III).

The 48 animals injected with normal tissue extracts which had been treated in comparable manner do not show any tumour lesions even after an interval of 126–212 days. BALLS[7] observed the appearance of the tumour in 28% of animals after injection of the supernatant fluid following centrifugation of the homogenate of normal tissue at 10,000 × *g* and he suggested a higher susceptibility to 'lymphosarcoma' development of the hosts used.

The lack of an obvious sedimentation of the tumour-inducing agent of *Xenopus laevis* even by means of very high speed centrifugations (150,000 × *g*, 2 × 60 min) is not in agreement with the size and density of animal viruses so far known[9].

In conclusion, considering the results of the experiments presented in this report, it can be stated that the *Xenopus laevis* 'lymphoid tumour' is caused by a subcellular agent, but its viral nature cannot be proved on the basis of information so far available (see also [3] and [10]).

Résumé. La «tumeur lymphoïde» de *Xenopus laevis* est transmissible par des filtrats acellulaires ainsi que par injection des surnageants de centrifugation des extraits de tissus cancéreux à des vitesses qui sédimentent les virus connus jusqu'à présent.

Table III. Incidence of the 'lymphoïd tumour' in *Xenopus laevis* following injection of filtrates of cancerous tissue extracts

Group	Filtrates (nm)	Autopsy in mean days after injection	No. of hosts with tumour Total No. of hosts	Hosts with tumour (%)
1	450	71	51/67	76
2	450	127	4/12 adults	33.3
3	450	118	20/34	58.8
4	220	65 and 102	18/34	53

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Some Characteristics of the 'Lymphoid Tumour' Inducing Agent of *Xenopus laevis*[1]

The 'lymphoid tumour' of the anuran amphibian *Xenopus laevis*[2] is transmissible by tissue transplantation[3–5] and by inoculation of cell-free extracts[6,7]. It had been suggested by BALLS[6] that this tumour might be caused by a virus. Animal viruses can be classified according to their nucleic acid type, their stability or instability in lipid solvents (ether or chloroform), their acid stability or lability, and their heat sensitivity[8].

In this report we present the results of experiments performed on the 'lymphoid tumour' extracts of *Xenopus laevis* to test their sensitivity to treatment with acid, ether, chloroform, and heat. We also try to determine whether or not the agent responsible for this tumour can be placed into one or the other group of animal viruses as classified by HAMPARIAN et al.[8].

Material and methods. The 'lymphoid tumour' used was of spontaneous origin and had been transplanted to a number of animals in order to produce a large amount of tumoral tissue. The method for preparation of cancerous tissue extracts has been described elsewhere[7]. In each experiment 0.1 ml of the extracts was injected into the dorsal lymph sac of 2–5-month-old *Xenopus laevis*. These were later examined to determine the incidence of the tumour.

Tests for ether sensitivity. Group 1. Cancerous tissue homogenate was centrifuged for 20 min at 2000 × *g* and the supernatant fluid was mixed with twice its volume of ethyl-ether at 4 °C. After 1 h the aqueous phase was separated by centrifugation at 2000 × *g* for 15 min and injected into 10 animals. Group 2 and 3. Cancerous tissue homogenates were centrifuged at 10,000 × *g* (30 min) and 130,000 × *g* (1 h) and the 2 supernatants were diluted, by addition of ethyl-ether, to 77% of their original concentration[7]. These mixtures were shaken at 4 °C for 24 h, the ether was allowed to evaporate, afterwards each solution was injected into a group of 10 animals.

Tests for chloroform sensitivity. Group 1. Supernatant fluid of a centrifugation of cancerous tissue homogenate at 10,000 × *g* (30 min) was mixed with 1/5 of its volume of chloroform at room temperature. After 30 min the chloroform was separated by centrifugation at 2000 × *g* for 15 min and the aqueous phase was injected into 17 animals. Group 2. The tumour extract was passed through

a 450 nm Millipore filter then mixed with twice its volume of chloroform at 4 °C. After 30 min the chloroform was separated as in Group 1 and the aqueous phase injected into 7 animals.

Tests for sensitivity at pH 3. The supernatant fluids, of a cancerous tissue homogenate centrifuged at 10,000 × *g* (30 min) and 130,000 × *g* (1 h) were acidified with N/10 hydrochloric acid to a pH of 3 ± 0.1. After 30 min the pH was raised to 7.1 ± 0.1 with N/10 NaOH and the 2 solutions were then injected into 27 and 21 animals respectively.

Tests for heat sensitivity. Group 1–3. The filtrate passed through a 450 nm Millipore filter and 2 supernatant fluids of centrifugations of tumour homogenates at 10,000 × *g* and 130,000 × *g* were heated to 56 °C in a water-bath for 30 min. After cooling, the solutions were injected into 16, 21, and 16 animals respectively.

Controls. In each experiment of the 4 different tests, samples of the corresponding untreated extracts were injected into a number of animals (see controls in the Table).

Results and discussion. The results of the 4 different tests performed on the tumour extracts are presented in the Table. The diagnosis of development of the 'lymphoid tumour' in the injected animals was based on both macroscopic and histological examination of the liver, spleen, and kidneys which have been shown to be the first three organs to exhibit tumoral lesions[9].

In the first group of each series of ether- and chloroform-treated extracts, the tumour incidence in the experimental animals was not different from the controls. This was probably due to the short exposure to, or an insufficient quantity of, the solvents used. However, in the second and the third groups of ether-treated, and in the second group of chloroform-treated extracts, the tumour incidence was clearly diminished.

The injection of the acid-treated tumour extract did not diminish the incidence of the tumour as compared with controls. In both series the animals developed the tumour in 100% of the cases. The injection of heat-treated tumour extracts did decrease significantly the incidence of the tumour in all 3 groups as compared with their controls.

The results presented in the Table lead to the conclusion that the agent of the *Xenopus laevis* 'lymphoid tumour', in tissue extracts, is sensitive to lipid solvents and heat (56 °C for 30 min) but remains stable after a 30 min treatment with pH 3.

Following the classification table of HAMPARIAN et al.[8] for animal viruses and considering the characteristics of the agent as presented in this report (sensitivity to lipid solvents combined with acid stability), it is not possible to find a known group of DNA or RNA viruses into

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Sensitivity of the 'lymphoid tumour' agent of *Xenopus* to different physico-chemical tests

Treatment (time)		Group	Tumoral extracts	Autopsy in mean days after injection	No. of hosts with tumour	Total No. of hosts	Hosts with tumour (%)	
					Experimental	Controls	Experimental	Control
Ether	1 h	1	2000 × *g* (20 min) supernatant	136	8/10	7/10	80	70
	24 h	2	10,000 × *g* (30 min) supernatant	44	1/10	7/7	10	100
	24 h	3	130,000 × *g* (1 h) supernatant	46	2/10	7/7	20	100
		Total			11/30	21/24	30.6	87.5
Chloroform	30 min	1	10,000 × *g* (30 min) supernatant	123	7/17	5/11	41	45
	30 min	2	Filtrate 450 nm (Millipore)	146	3/7	10/11	43	91
		Total			10/24	15/22	41	68
pH 3 ± 0.1	30 min	1	10,000 × *g* (30 min) supernatant	45	27/27	7/7	100	100
	30 min	2	130,000 × *g* (1 h) supernatant	46	21/21	7/7	100	100
		Total			48/48	14/14	100	100
Heat (56 °C)	30 min	1	Filtrate 450 nm (Millipore)	89	1/16	16/19	6.3	84.2
	30 min	2	10,000 × *g* (30 min) supernatant	47	6/21	13/13	28.5	100
	30 min	3	130,000 × *g* (1 h) supernatant	47	2/16	7/7	12.5	100
		Total			9/53	36/39	17	92.3

which the *Xenopus laevis* 'lymphoid tumour' agent could be fitted.

In addition, our attempts to demonstrate the presence of 'viral particles' in tumour cells by electron microscopy[9] and the search for a 'viral agent' in the extracts prepared from the affected tissues and submitted to different methods of fractionation (unpublished data) have so far been unsuccessful.

The results mentioned above are not in complete agreement with the observations on this tumour agent made by BALLS and RUBEN[10]. While our unsuccessful search for viral particles does not suggest a viral origin of this tumour, BALLS and RUBEN[10,11] have advanced a possible viral etiology mostly based on their experiments with ultracentrifugation and filtrations. More experiments ought to clarify the nature of the agent and the etiology of the 'lymphoid tumour' of *Xenopus laevis*.

Résumé. L'infectivité des extraits tissulaires de la «tumeur lymphoïde» de *Xenopus laevis* diminue après traitement à l'éther, au chloroforme, et à la température de 56 °C, tandis qu'elle reste inchangée après traitement au pH acide. Ces caractéristiques ne permettent pas de placer l'agent tumoral parmi les virus des animaux connus à l'heure actuelle.

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Änderung der Wachstumskinetik von Ehrlich-Aszitestumoren verschiedener Ploidie nach Röntgen-Bestrahlung

Der diploide ($EAT_{dipl.}$) beziehungsweise hyperdiploide (ELD) und der hypertetraploide (ELT) Ehrlich-Aszitestumor zeigen ein unterschiedliches Wachstumsverhalten[1,2]. Darüber hinaus wurde festgestellt, dass sich der Generationszyklus des $EAT_{dipl.}$ und ELT nach Gabe von Cyclophosphamid (100 mg/kg) in verschiedenem Masse verändert[3]. In der vorliegenden Arbeit wurde untersucht, ob bei diesen verschieden-ploiden Tumorzellstämmen auch Unterschiede in der Beeinflussung des Generationszyklus und seiner Teilphasen nach Röntgen-Bestrahlung nachweisbar sind.

Untersuchungsgut und Methodik. Männlichen NMRI-Mäusen (Institut für Versuchstierzucht Hannover; 23–25 g; Brovo-Versuchstierfutter) wurden ca. 18×10^6 Zellen des $EAT_{dipl.}$ beziehungsweise ELT i.p. inokuliert. Am 5. Tag nach Inokulation wurde eine Röntgen-Bestrahlung der Tumor-Mäuse durchgeführt (500 R, 110 R/min, 200 kV, 10 mA, 0,5 mm Cu; Röhreneigenfilterung 0,1 mm Cu; 3 cm Glastubus, FHA 25 cm; rein abdominales Bestrahlungsfeld, Siemens Stabilipyan). 24 h nach Röntgen-Bestrahlung (Rö) wurde die Häufigkeit der absterbenden beziehungsweise nekrotischen Zellen (Nekroseindex) durch Lissamin-Green-Färbung nach HOLMBERG[4] ermittelt. Der Generationszyklus der $EAT_{dipl.}$ beziehungsweise ELT-Zellen wurde durch das Doppelmarkierungsverfahren[5,6] (DMV) und nach der %-markierte-Mitosen-Methode[7] (%-MM-Methode) analysiert (Näheres zur Methodik siehe bei[2]).

%-MM-Methode. 62 $EAT_{dipl.}$-Mäuse und 60 ELT-Mäuse erhielten am 5. Tag nach Inokulation und 1 h nach Rö einmalig Thymidin-Methyl-^{3}H i.p. (50 µCi; 2,0 Ci/mmol, NEN Corp. USA). Tötung der Tiere in gestaffelten Zeitintervallen (2–44 h) nach ^{3}H-TdR-Injektion. Bestimmung des Prozentsatzes der markierten Mitosen auf von Aszitesausstrichen hergestellten Autoradiogrammen (ARG) mit K2-Emulsion (Ilford, London; Exposition 8 Tage).

Doppelmarkierungsverfahren. 7 mit $EAT_{dipl.}$ und 11 mit ELT beimpfte Mäuse erhielten am 5. Tag nach Inokulation und 4 h nach Rö eine erste i.p.-Injektion von Thymidin-2-^{14}C (10 µCi; 30 mCi/mmol, NEN Corp. USA) und 1 h später eine zweite i.p.-Injektion von Thymidin-Methyl-^{3}H (100 µCi; 2,0 Ci/mmol). Auf den von Aszitesausstrichen hergestellten ARG (G5-Emulsion, Ilford, London; Exposition 2–5 Tage) wurde das Verhältnis aller ^{14}C-markierten Kerne (mit und ohne ^{3}H) zu den rein ^{3}H-markierten Kernen und der ^{3}H-Markierungsindex (^{3}H-I) bestimmt. Aus dem Häufigkeitsverhältnis der unterschiedlich markierten Kerne (= v) und dem ^{3}H-I wurden die DNS-Synthesezeit (T_s) und die Generationszeit (T_c) berechnet, nachdem G2 + M (= prämitotische Ruhephase + Mitosedauer) durch die %-MM-Methode bestimmt worden war (Berechnungsverfahren siehe bei[2]).

^{3}H-TdR-Dauermarkierung. An je 4 mit $EAT_{dipl.}$ beziehungsweise ELT beimpften Mäusen (5. Tag nach Inokulation, 2 h nach Rö) wurden Dauermarkierungsversuche mit wiederholter Gabe von ^{3}H-TdR (i.p.-Injektion von je 40 µC pro Maus; 2,0 Ci/mmol) durchgeführt. Die einzelnen Injektionen erfolgten im Zeitabstand von 8 h über eine Versuchsdauer von 32 h. Bestimmung des Prozentsatzes der markierten Tumorzellen auf K2-ARG.

Tumorzellzählung. 72 h nach Rö wurde für den $EAT_{dipl.}$ und den ELT die Gesamtzellzahl des Tumoraszites bestimmt und mit den Werten für den unbestrahlten Tumor verglichen.

Ergebnisse. Tabelle 1, b, enthält die wesentlichen Ergebnisse. Es fand sich 24 h nach Rö für die $EAT_{dipl.}$-Zellen ein Nekroseindex (LG-Index) von 12%, für die ELT-Zellen von 35%. Der Generationszyklus der beiden Tumorzellstämme zeigte unterschiedliche Veränderungen: Beim $EAT_{dipl.}$ war die DNS-Synthesezeit T_s nach Rö

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