Transmission of the 'Lymphoid Tumour' of *Xenopus laevis* by Injection of Cell-Free Extracts[1]

The 'lymphoid tumour' in *Xenopus laevis* was first described by BALLS[2]. Later we determined the spontaneous incidence of the tumour which varied during the 4 years of observation between 1.5–4.2% for wild animals, and 2.2–7.1% for laboratory bred animals[3]. Localized tumours are first and mainly observed in the liver, spleen and kidneys[3]. The tumour is transmissible to healthy *Xenopus* by tissue transplantation[4–6].

In this paper we report results of the transmission of the tumour after injection of cell-free extracts prepared by filtration and high speed centrifugation of cancerous tissue homogenates. As will be shown, our results differ in some aspects from those previously published by BALLS and RUBEN[7,8].

Material and methods. The extracts were prepared by homogenizing the liver of tumour bearing animals in phosphate buffer solution of 0.01 *M* to a final concentration of 20–30% (W/V). These homogenates were clarified by centrifugation at 2000 × *g*. The resulting supernatant fluids were for certain experiments again centrifuged at 10,000 × *g* for 30 min in a Spinco model L ultracentrifuge, rotor SW 40. The supernatants of centrifugation at 2000 × *g* and 10,000 × *g* were used as basic material in all experiments described below.

Xenopus laevis, of both sexes and 3–4 months of age (20–30 mm mouth-cloaca), were injected into their dorsal lymph sac with 0.1 ml of the extracts. In one experiment adults were also used but received 0.5 ml of the extract (see filtration experiment, group 1).

Centrifugation. Groups 1 and 2. Supernatant fluids from 4 different tumour homogenates, centrifuged at 2000 × *g* and 10,000 × *g*, were injected into 47 and 34 immature animals respectively. Groups 3, 4 and 5. In 3 different experiments, using the supernatant fluids from the centrifugations at 10,000 × *g*, further centrifugations at 130,000 × *g* (rotor SW 39) were carried out for 45, 60 and 150 min. The 3 different supernatants thus obtained were injected into 3 groups of 10, 7 and 5 immature animals. Groups 6 and 7. The supernatant fluid from a centrifugation at 10,000 × *g* was further centrifuged at 150,000 × *g* (rotor SW 39) for 60 min. The top 2/3 fraction of this supernatant was again centrifuged at 150,000 × *g* for another 60 min, and the top 1/3 fraction of the last supernatant was injected into 10 immature animals. The pellet of the first centrifugation at 150,000 × *g* was suspended in a phosphate buffer solution and injected into 11 immature animals. Group 8. The supernatant fluid of a centrifugation at 10,000 × *g* was further centrifuged at 130,000 × *g* for 45 min. $1^1/_2$ ml of this supernatant was layered over successive layers of 10% and 20% sucrose, followed by centrifugation at 150,000 × *g* for 120 min. This procedure allowed us to distinguish 5 different layers, each of which was injected into 5 groups of 11, 11, 8, 7 and 8 immature *Xenopus* respectively (see Table II).

Filtration. These experiments were carried out by Millipore filtration under pressure. Groups 1 and 2. The supernatants collected after centrifugation of cancerous tissue homogenates at 2000 × *g* were passed through 450 nm filters. The filtrates were then injected into 67 immature (group 1) and 12 adult *Xenopus* (group 2). Groups 3 and 4. The supernatants of centrifugation of cancerous tissue homogenates at 10,000 × *g* were passed through a 450 nm filter. Parts of these filtrates were injected into 34 immature animals while the remaining parts were in turn run through a 220 nm filter and then injected into 34 immature animals.

Control. The homogenate of normal liver, its supernatant from centrifugation at 2000 × *g*, and the filtrate of the latter (450 nm) were injected into 16 animals each.

Results and discussion. The diagnosis of development of the tumour was based on microscopic examination of the liver, spleen and kidneys of all injected animals. The results of the centrifugation experiments of group 1–7 are presented in Table I. The development of the tumour is observed in 123 out of 124 animals. In groups 3–6, in which the supernatant fluids of high speed centrifugation (cell-free) were used, the incidence of the tumour is as high as in groups 1 and 2, in which a low speed centrifugation was performed and tumour cells might have been present. In none of these groups is a very obvious sedimentation of the agent observed. Nevertheless, a difference in the interval of appearance of the tumour is noted between the 2 groups of animals injected either with the pellet of the first centrifugation at 150,000 × *g* (1 h) or with the supernatant fluid of the second centrifugation

Table I. The incidence of the 'lymphoid tumour' of *Xenopus laevis* following injection of supernatant fluids and the pellet of centrifugation of cancerous tissue extracts

Group	Supernatant fluids (× *g*)	Duration of centrifugation (min)	Autopsy in mean days after injections	No. of hosts with tumour Total No. of hosts	Hosts with tumour (%)
1	2,000	20	37[a] and 50[a]	47/47	100
2	10,000	30	29[a] and 135[a]	33/34	97
3	130,000	45	62	10/10	100
4	130,000	60	47	7/7	100
5	130,000	150	93	5/5	100
6	150,000	2 × 60	106	10/10	100
7	Pellet of 150,000 × *g*	60	42	11/11	100

[a] Mean in different experiments.

Table II. Results of injection of different fractions of ultracentrifugation of cancerous tissue extract on sucrose solutions (group 8)

Fractions from top to bottom	Autopsy in mean days after injection	No. of hosts with tumour Total No. of hosts	Hosts with tumour (%)
1	105	7/11	63.6
2	109	9/11	81.8
3	90	7/8	87.5
4	86	7/7	100
5	76	8/8	100

[1] This investigation was supported by the Fonds National Suisse pour la Recherche Scientifique No. 4411.

[2] M. BALLS, Cancer Res. *22*, 1142 (1962).

[3] I. HADJI-AZIMI et M. FISCHBERG, in preparation.

[4] M. BALLS, Cancer Res. *24*, 4 (1964).

[5] I. HADJI-AZIMI et M. FISCHBERG, Revue suisse Zool. *75*, 706 (1968).

[6] I. HADJI-AZIMI and M. FISCHBERG, in preparation.

[7] M. BALLS, Ann. N.Y. Acad. Sci. *126*, 256 (1965).

[8] M. BALLS and L. N. RUBEN, Cancer Res. *27*, 654 (1967).

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

I. HADJI-AZIMI[11]

Station de Zoologie expérimentale, Université de Genève, CH-1224 Genève (Switzerland), 30 January 1970.

[9] C. A. KNIGHT, in *Protoplasmologia IV* (Springer Verlag, Berlin 1963), p. 13.
[10] I. HADJI-AZIMI, Experientia *26*, 895 (1970).
[11] I wish to thank Professor M. FISCHBERG for his interest in this work and the facilities he provided.

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