

# Renal Adenocarcinomas Promoted by Crowded Conditions in Laboratory Frogs<sup>1</sup>

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## SUMMARY

Spontaneous renal adenocarcinomas in the frog are presumed to be caused by a virus but not naturally transmitted between adult frogs. However, we have observed a high incidence of renal tumors in frogs maintained in the laboratory under crowded conditions in contrast to frogs housed under relatively uncrowded conditions. These results suggest that under laboratory conditions natural transmission or an unknown environmental factor(s) is important in the epidemiology of the frog renal adenocarcinoma.

High incidences of spontaneous renal tumors have been reported by the Raffertys (10) in leopard frogs (*Rana pipiens pipiens*) that were maintained under crowded conditions in the laboratory for periods ranging from 3 to 10 months. These incidences (20–50%) are significantly higher than the incidence that Lucké (5) found in newly caught frogs (2.7%) or in frogs that he maintained in the laboratory for a year or more (6.7%). Subsequent studies by Rafferty (7) have shown that high environmental temperature and large body length are important factors in promoting high incidences of renal tumors in laboratory-maintained frogs and that the temperature factor, at least, can account for the differences observed by several investigators. Since the incidence of renal tumors in isolated frogs was found to be the same as that in frogs maintained under crowded conditions (8, 11), Rafferty (9) has suggested that natural transmission between adult frogs is not a factor in spontaneous tumor development and that, if a virus is involved, it may act as an infective agent earlier in the life cycle of frogs.

Stimulated by Rafferty's initial findings (10), we maintained a colony of adult leopard frogs under laboratory conditions primarily to obtain a dependable supply of renal tumors for nuclear-transplantation studies (1, 3). In addition, we designed the experiment to examine to what extent the degree of crowding influences the incidence of spontaneous tumor formation. In October, 1961, when this experiment was initiated, information on this point was not available.

## MATERIALS AND METHODS

All frogs used in this experiment were collected by us from the fields bordering the Mud Creek and Swanton River areas in the vicinity of Alburg, Vermont. These

collecting areas include part of the Lake Champlain region, long used as the major source of *Rana pipiens pipiens*. At the time of capture, no palpable renal tumors were detected in frogs obtained from either of the 2 collecting areas. On arrival at our laboratory the frogs were separated according to sex, then distributed into groups of 1, 2, 3, 4, or 6 individuals. The dorsal pigment pattern of all frogs was photographed, and the negatives were used throughout the experiment as a means of identifying each individual frog. Before the experiment was begun the frogs were housed in glass aquaria and kept in spring water in the refrigerator at 4°C for 4 months. Following this storage period the experiment was initiated and the frogs were placed in a laboratory room at 22° ± 2°C. At this time the frogs were again palpated for renal tumors, but none was detected. The body length from rostrum to pubis was measured, and each frog received 3000 units of penicillin i.p. to prevent the possibility of deaths due to bacterial infections (7). All the frogs were housed in 3.5-gal rectangular tanks constructed of glass sides and a slate floor. Each tank received 500 ml of dechlorinated tap water once a week, and the frogs were fed a diet of crickets, earthworms, and/or beef liver twice a week. The animals were maintained in the laboratory for 34 months. Three and one-half months after the initiation of the experiment the animals were palpated and administered a 2nd injection of penicillin. Thereafter, they were palpated monthly. The body length of individuals that died or were sacrificed for use in other experiments was measured; then the animals were autopsied and examined for the presence of tumors.

## RESULTS

Among the 93 frogs that composed this experiment, 25 developed renal tumors. Twenty-two renal tumors were examined, histologically, and all conformed to the histopathology of the frog renal adenocarcinoma as described by Lucké (4). Eighteen of the tumor-bearing frogs

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TABLE 1

THE OCCURRENCE OF SPONTANEOUS KIDNEY TUMORS IN FROGS MAINTAINED IN THE LABORATORY AT 22° ± 2°C FOR 34 MONTHS

No. OF FROGS/ TANK	No. OF TUMOR-BEARING FROGS IN EACH OF 24 TANKS								TOTALS	
									No. of frogs	No. of tumor- bearing frogs
1	0	0							2	0
2	0	0	0	1	1				10	2
3	0	0	0						9	0
4	0	0	0	1	1	2			24	4
6	0	1	2	2	3	3	4	4	48	19
Total									93	25

TABLE 2

MEAN BODY LENGTH AND MEAN LABORATORY AGE OF FROGS AT DEATH

CONDITION OF KIDNEY	POPULATION SIZE <sup>a</sup>	TOTAL NO. OF FROGS	BODY LENGTH (mm) <sup>b</sup>		LABORATORY AGE (MONTHS)	
			♂	♀	♂	♀
Tumors	Crowded	19	71.1	75.2	13.6	14.3
	Less crowded	6	71.8		13.3	
No tumors	Crowded	29	73.4	84.0	17.8	23.2
	Less crowded	39	73.9	82.9	20.2	24.7

<sup>a</sup> Crowded, 6 frogs/tank; less crowded, 1-4 frogs/tank.

<sup>b</sup> In Rafferty's study (8) the average body length in which a relatively high incidence of spontaneous renal tumors occurred was: ♂, 72.9 mm; ♀, 80.0 mm.

possessed bilateral renal tumors, which ranged in size from 10 x 20 x 4 mm to 35 x 38 x 20 mm; 7 of these frogs also had tumors in the liver, lungs, urinary bladder, or testes. The 7 other renal tumors were unilateral; 5 were large (from 9 x 10 x 5 mm to 26 x 38 x 25 mm) and 2 were small (2.5 x 3.0 mm and 3.0 x 5.0 mm). Two of the frogs with large unilateral tumors also had tumors in the lungs and ovary, respectively. Forty-nine frogs that died during the course of the experiment did not possess macroscopically detectable renal tumors. At the termination of the experiment 20 frogs remained, and 1 of these had a small unilateral kidney tumor.

The influence of crowding in promoting a high incidence of renal tumors is shown in Table 1. Approximately the same total number of frogs was housed in groups of 1-4 or 6 (45 and 48, respectively); however, 19 tumors arose in frogs that were housed in groups of 6, and only 6 tumors occurred in frogs maintained under less crowded conditions. Chi-square analysis of this difference was highly significant ( $P < 0.01$ ). Also characteristic of frogs under crowded conditions was the multiple occurrence of tumors within 1 tank; for example, most of the crowded tanks contained 2-4 tumor-bearing frogs, whereas only 1 tank of the less crowded group contained more than 1 tumor-bearing frog.

Previously, Rafferty (7, 8) found that a relatively high incidence of spontaneous renal tumors occurs in frogs of

large body length. Body length was taken as an indication of relative age. In our laboratory frogs, the mean body length of all groups of frogs was about the same as or greater than the mean body length of frogs that Rafferty found to possess a high incidence of tumors (see Table 2). Furthermore, those frogs which did not have renal tumors had a slightly greater mean body length than frogs with renal tumors. Equally important is the fact that the mean laboratory age of frogs in the less crowded group was about the same as or greater than the mean laboratory age of frogs in the crowded group. In addition, frogs that did not bear renal tumors lived longer than tumor-bearing frogs. Thus, it is not likely that laboratory age and body length alone have promoted a higher incidence of renal tumors among crowded frogs.

The following additional findings emerged from our present study. First, although the colony was maintained for 34 months, 72% of the tumors arose during the 1st 14 months. Second, at least 20% of the frogs remained refractory to spontaneous tumor formation. This indicates that some frogs possess a natural resistance to tumor formation, even under conditions that favor a high rate of tumor development. Third, no striking difference in percentage of tumor-bearing frogs was observed between the sexes (females, 33%; males, 25%).

DISCUSSION

The present study was conducted for 34 months, during which time 27% of the frogs developed renal tumors. Of these tumors, 76% occurred in frogs that were housed in crowded groups. Contrary to previous reports (5, 8, 11), the present data suggest that under crowded conditions in the laboratory natural transmission among mature frogs is an important factor in the epidemiology of the frog renal adenocarcinoma. Support for this thesis comes from the fact that the occurrence of renal tumors in more than 1 frog/group was found almost exclusively under crowded conditions.

Although electron microscopic studies have demonstrated a virus in the frog renal adenocarcinoma, it is not known whether this virus is the oncogenic agent (2, 6). Since the etiology of this tumor is not definitely established, our present results are open to a number of interpretations. An infective agent may under crowded conditions be transmitted from one frog to another *via* direct contact or through respiratory waste products or excreta. On the other hand, a noninfective factor in frog waste products may accumulate to a threshold level under crowded conditions. When recycled through the kidneys, these products might irritate the cells of the kidney tubules. Such a physiologic stress might either induce tumor formation *per se* or promote the formation of tumors in frogs already infected at the egg or tadpole stage, the time at which Rafferty (9) proposes that the tumor virus enters as a latent infective agent.

It should be pointed out that some of the conditions under which this experiment was conducted differed from those used by Rafferty (8) and Roberts (11). Before being brought to the temperature of the laboratory, our frogs were stored at 4°C for 4 months, whereas the frogs used by Rafferty and Roberts were placed at laboratory tem-

perature immediately after capture. The present study was carried out over a 34-month period, but previous studies were conducted for only 4 and 8 months (11, 8). Our frogs at weekly intervals were given 500 ml of tap water, dechlorinated with sodium thiosulfate (Dr. R. R. Humphrey, Department of Zoology, Indiana University, personal communication). In Rafferty's experiments the crowded frogs received about 2000 ml of tap water daily, and the tanks were arranged so that a constant interchange of small amounts of water occurred; his isolated frogs were given about 100 ml of tap water at about daily intervals. Our frogs were fed a diet of crickets, earthworms, and/or beef liver twice a week; however, Rafferty and Roberts maintained their animals on a diet of mealworms once and twice a week, respectively. The significance of these differences in promoting a high incidence of renal tumors in laboratory-maintained frogs is unknown.

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