oxygenation (*5*). A series of preliminary observations over a 4-week period had established that no apparent harm resulted from intraperitoneal injections of the test substances.

The results with the first three groups of animals are shown in Fig. 1. The survival rate of the group of rats treated with $NaHCO_3$ was significantly greater ($P < .001$) than that of the group treated with saline or THAM. Most of the deaths occurred during the first 4 days of exposure to O_2 and were accompanied by extensive pulmonary congestion. The animals treated with $NaHCO_3$ had less dyspnea in the early period of exposure and a lower incidence of fatal pulmonary damage. The inadequate protection observed with THAM treatment in these experiments may be due to the compound's hypoglycemic action. The additional stress of hypoglycemia in severely ill animals could mask any protective effect of this titrating agent. Treatment with $NaHCO_3$ did not prevent testicular degenerative changes which were present in the surviving animals that were killed after 30 days (Fig. 2). These changes appeared in the untreated animals as early as 2 days after exposure to oxygen. All the rats treated with chlorpromazine died within 3 days of oxygen inhalation.

The only known effect of $NaHCO_3$ administration is an increase in the content of base in body fluids. The mechanism by which this effect might increase the survival rate of animals exposed to high oxygen tensions remains to be determined. Such a protective action, if also demonstrated in man, might have therapeutic applications in the case of patients inhaling enriched oxygen mixtures at atmospheric or elevated pressures. (*6*).

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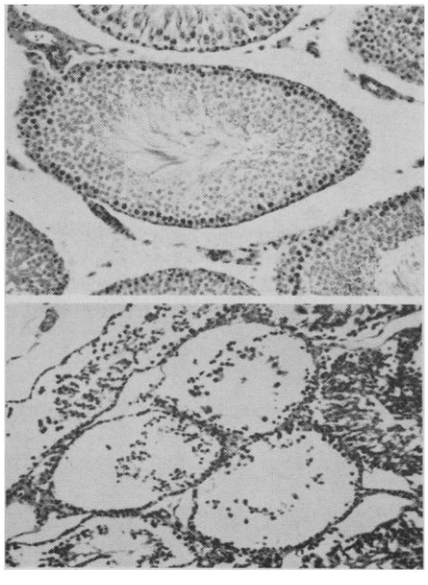

Fig. 2. (Top) Normal seminiferous tubules of a rat. (Bottom) Changes in the seminiferous tubules of a rat treated with $NaHCO_3$ while breathing 95- to 98-percent O_2 for 30 days.

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Spontaneous Kidney Tumors in the Frog: Rate of Occurrence in Isolated Adults

Abstract. *Kidney tumors in the frog are thought to be virus-caused and naturally transmitted. The present report indicates that natural transmission between mature adults is not a factor in spontaneous tumor development and implies primary infection at earlier stages if a virus is indeed involved. Parasitism and nutritional state appear to be of little significance.*

The evidence favoring the view that the Lucké tumor of *Rana pipiens* is virus-caused has required re-examination because of the findings that (i) under room-temperature conditions, large, well-fed frogs develop spontaneous tumors at the unexpectedly high incidence of 20 to 50 percent, and (ii) under such conditions this incidence is essentially unaffected by injection of tumor extracts, although formation may be accelerated initially (*1–3*) and more striking differences in response may be observed when the promoting conditions are not fulfilled (*4, 5*). However, since about 2.7 percent of large commercial frogs from northern Vermont bear small tumors at the time of arrival in the laboratory (*4*), it is evident that transmission between adults under the promoting conditions might explain both phenomena. Lucké was unable to show clearly that the presence of known tumor-bearing frogs in the same tanks with presumably normal frogs influenced the spontaneous incidence (*4*). It has become evident in recent years, however, that control groups in such experiments might be exposed to as effective a level of virus as experimental groups, present in the urine of frogs bearing tumors too small for detection, or in that of infected but otherwise normal individuals. Roberts showed that isolation of frogs did not alter the spontaneous incidence over a 4-month period (*3*); however, she used frogs obtained from a dealer and mailed in crowded containers under conditions which would tend to encourage possible cross-infection. Since *Rana pipiens* is a solitary animal, close contact between individuals in the field is unusual, except for brief pairing during the spawning season, and possible huddling during winter hibernation. Hence it became apparent that frogs isolated in the field at the moment of capture might subsequently exhibit lowered rates of spontaneous tumor formation. Accordingly, 216 frogs were captured in the vicinity of North Hero Station, Vermont, in August 1962. All individuals were taken in open, grassy fields which usually contained little or no standing water. As each frog was located it was seized with a sterile, disposable plastic glove, and immediately transferred to a sterile plastic bag. While contained in the bag the frog was sexed and its length from rostrum to pubis was recorded. In addition, each frog was individually identified for subsequent references by sketching the highly individual dorsal spots. Each animal was then matched as closely as possible with another alike in sex, length, and field of capture. From each pair, one randomly chosen individual was then transferred to an aquarium tank (crowded control) and the other sealed in a new, wide-mouth glass jar of 1.9 liters capacity (isolation) without direct handling or any contact with possible sources of contamination. The metal jar tops were provided with two short lengths of stainless-steel tubing soldered near the rim on opposite sides. These were subsequently used as inlet-outlet openings for water changes and feeding, and ef-

fectively prevented splash from the jars. The tops were sealed to the jars by first painting the threads with rubber cement. Water was changed on 6 or 7 days a week. With the isolation jars, water was poured off through the outlet tube and fresh water (about 100 ml) added from a tap. In the crowding groups each of the ten tanks used contained 11 or 12 frogs. Water was changed daily as part of the animal house routine, and the tanks were arranged so that constant interchange of small amounts of water occurred. Each tank contained about 2 liters of water. Beginning in the third month of observation, all frogs were fed weekly with live mealworms (*Tenebrio molitor*) dropped into the two types of containers at the rate of about six per frog.

In order to determine how many tumors of subpalpable size might be expected among observation animals at the time of capture, 61 large male and 60 large female frogs were captured in the same areas a few days later and immediately killed for autopsy. Surprisingly, none bore even small tumors. In the observed groups the first tumor became palpable after 2 months, and the next two tumors not until 2 months later. It was concluded that only a few individuals, or perhaps none, bore tumors at the beginning of observation.

Observation was begun with 108 animals in each group, consisting of 64 males and 44 females. Isolated males ranged between 68 and 81 mm in body length, except for a single individual of 65 mm; crowded males ranged between 67 and 80 mm, except for one individual measuring 88 mm; average lengths for the two groups were 72.4 and 72.9 mm, respectively. Isolated females measured between 75 and 91 mm, compared with 75 to 92 mm (except for one frog measuring 71 mm) in the crowded females. Averages for these groups were 79.9 and 80.0 mm, respectively. Previous observations had determined that relatively high spontaneous incidence is to be expected in frogs of these size ranges (*2*).

Frogs in the crowded groups were palpated for tumors each month, and all animals which died in either group were autopsied. At the end of 8 months the remaining frogs were killed for autopsy and scored for the presence of a kidney tumor, kind and distribution of parasitic worms, general condition, and body weight.

Table 1 gives the results of the tumor survey. In constructing this table, I accounted for frogs dying without tumors by computation of cumulative incidence for each month. Since isolated frogs could not be palpated, the rate of tumor formation necessarily appeared to lag in their case; hence only the cumulative incidence figure for the eighth month is shown. It is evident that the incidence for both groups is essentially identical at 23.8 percent among isolated and 23.5 percent among crowded frogs. Of the 44 tumors found, 30 occurred in males. It was not possible to keep more than a few of the tumor-bearing frogs from the crowded groups until they died from the malignancy, since this tumor tissue was needed for other experiments. However, several cases of metastasis were seen in frogs in isolation jars which died with tumors. The tumors, like those in the crowded groups, often grew to large size, and there was no evidence that the character of tumors arising in the two groups was in any way different. Histological examination of several examples from each group showed them to be adenocarcinoma of the typical form described by Lucké (*4*).

Table 1. Incidence of spontaneous kidney tumors in frogs observed for 8 months.

Item	Isolated frogs	Crowded frogs
Starting number	108	108
Dead without tumors (No.)	32	8
Frog observation periods (No.)	84	102
Frogs with tumors (No.)	20	24
Final incidence (%)	23.8	23.5

Of isolated frogs, 39 of 68 (57.4 percent) remaining at the end of observation presented evidences of helminth infestation, while the rate was 41 of 88 cases (46.6 percent) for the crowded controls. The lung was the most common site of infestation, and trematodes greatly predominated over nematodes. There was no evidence of present or recent renal infestation, although a few kidneys bore very small infiltrated nodules which are believed to be the remains of resorbed worm sites. Of 11 small tumors found in these animals, six occurred in uninfested frogs. It is concluded that helminth infestation is not obviously related to tumor formation.

At termination, isolated frogs weighed less on the average than crowded frogs of the same sex, although lowered body weight was only slightly associated with parasitism. During the course of the experiment there was no change in body length in any group. At termination, isolated males weighed 18.7 g versus 23.6 g for crowded males; for females, corresponding averages were 23.4 and 32.6 g. Although weights were not recorded at the beginning of the experiment, final weights were low and most frogs were thin. Apparently, nutritional conditions were not optimal, a conclusion also indicated by a rapid increase in deaths of incidental cause (but without evidence of bacterial disease) in the last 3 months. Most of these deaths occurred in isolated frogs, which also showed lower average weights; consequently, it is concluded that isolated frogs were in poorer nutritional condition. However, the 11 frogs with small tumors found on autopsy showed weights which agreed well with the averages for their experimental groups. These observations all indicate that nutrition is relatively unimportant in tumor development, although Lucké showed that ample feeding greatly increases formation of metastases in frogs already bearing tumors (*6*).

The tumor is believed to be viral in etiology, partly because rate differences in tumor formation are associated with injection of cell-free tumor extracts under some conditions (*4*, *5*).

It is concluded, however, that virus transmission among adult frogs is not a factor in tumor development, although this finding does not rule out possible infection *in ovo*, or infection of larval or young adult stages. Nutritional factors seem to be of minor importance in formation of the tumors, although of demonstrated significance in their subsequent development. Finally, worm infestation either has no bearing on tumor formation, or is not related to tumor formation in any simple way (*7*).

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