to predict total body fatness from a measurement (say, specific gravity) made on an eviscerated carcass with 67-percent fatness, the error incurred would be +6.5-percent fatness. The error would be progressively smaller on leaner animals.

These data suggest the possibility that adipose depots associated with the viscera achieve a limiting value in the region of 28-percent fatness beyond which additional fat may not be accommodated, leaving further fat accumulation to depots associated with the eviscerated carcass. This hypothesis is tested in Fig. 2, where visceral fatness is plotted against total body fatness.

Inspection suggests a complex function with a "break." In Fig. 1 the intercept (24.4 percent) of $y = 3.9 + 0.84\ x$ with $y = x$ is seen to coincide with a small interruption in the data. Using this as the "break" point, two different regression lines were calculated, namely, one for individuals with total body fatness below 24.4 percent and another for those above. The first intercepts the ordinate close to the origin and has a slope (0.98) not statistically different from 1.00. The slope of the second (0.04) is not statistically different from that of a line parallel to the abscissa. The scatter is greater than that in Fig. 1, particularly in the obese mice that had free access to food, where the range of ordinate values appears to be greater than can be accounted for solely by methodological errors. In spite of its genetic purity this group shows an increased biological variability in visceral fatness which we are unable to explain. The obese mice on a restricted diet (nine individuals to the left) have a more restricted range of ordinate values.

In general, Fig. 2 supports our initial hypothesis that in the region of 24- to 28-percent total body fatness, there is a limiting value, below which visceral depots participate proportionately in fat storage, and above which additional fat is not accommodated in these depots. In spite of the statistical finding that the slope of the regression curve above the break is not significantly different from zero, an element of doubt is introduced by the wide range of ordinate values in the individuals at the right. Because the one very low value ($y = 10.6$) may exert an inordinate weight, we have recalculated the regression line omitting that point. The slope becomes 0.105 and there are still 85 chances out of 100 that our sample came from a population with a slope of zero.

It should be noted that the viscera constitute less than one-fifth of the total body weight in these animals. Consequently, the difference between proportionate fat storage and no fat storage by the viscera causes only a small alteration in the slope of the function in Fig. 1.

Finally, it should be re-emphasized that our findings apply only to female mice of the genetically obese strain. The desirability of experimental verification in other populations is evident (*6*).

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17 July 1963

Sodium Bicarbonate: Increase in Survival Rate of Rats Inhaling Oxygen

Abstract. *The injection daily of 50 milliliters of 0.15*M *sodium bicarbonate per kilogram of body weight significantly decreased the mortality rate of rats inhaling 95- to 98-percent oxygen. This treatment did not prevent the occurrence of degenerative changes in the germinal epithelium of the testes.*

It has been shown previously (*1*) that intraperitoneal injection of sodium bicarbonate or tris (hydroxymethyl) aminomethane (THAM) significantly delays the appearance of convulsions in mice exposed to high pressures of oxygen (up to 4.7 atmospheres absolute). Furthermore, these buffers increased significantly the 24-hour survival rate after the animals were removed from a high oxygen environment.

Elevated mortality rates and lung damage (*2*) are observed when animals are exposed for several days to concentrations of oxygen greater than 70

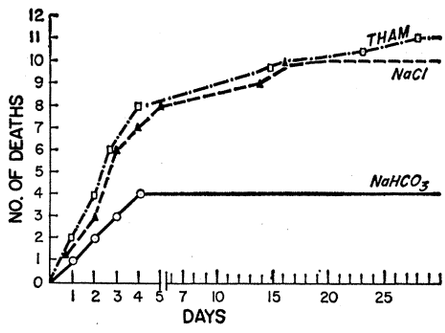

Fig. 1. Mortality rate during one month of rats breathing 95- to 98-percent O_2. Most of the deaths occurred in the first 4 days. There was a significant increase in survival rate of the animals treated with $NaHCO_3$.

to 80 percent. Testicular changes have been noted in rodents exposed to high pressures of oxygen (*3*) or exposed for several weeks to pure oxygen (*4*). The present experiment was designed to assess the effects of THAM and $NaHCO_3$ on survival and on changes in the gonads of rats exposed to 95- to 98-percent oxygen for a period of 4 weeks.

Male albino rats (Sherman strain) weighing 180 to 270 g were placed in glass aquariums which were then sealed and flushed with oxygen. A continuous flow of 3 to 4 liters of oxygen per minute was maintained. Gas was removed from the aquariums with a blower and circulated through containers filled with soda lime and silica gel before it was returned to the tanks. This insured the removal of carbon dioxide and excessive moisture from the system. Samples of the gas from the aquariums were analyzed frequently with an infrared carbon dioxide analyzer and a Beckman oxygen analyzer. Water and food were constantly available.

Three groups of 12 rats were studied. The first group was injected intraperitoneally with 50 ml of 0.15*M* $NaHCO_3$ per kilogram of body weight. The second group was injected intraperitoneally with an equal volume of 0.3*M* THAM titrated to *p*H 8.55 with HCl. This titration to a lower *p*H is necessary to avoid severe peritoneal irritation. The control group received an equal volume of isotonic saline. A fourth group (six rats) was similarly injected with an equal volume of chlorpromazine solution (15 mg/kg). Chlorpromazine protects against convulsions and increases the survival rate of animals exposed to hyperbaric

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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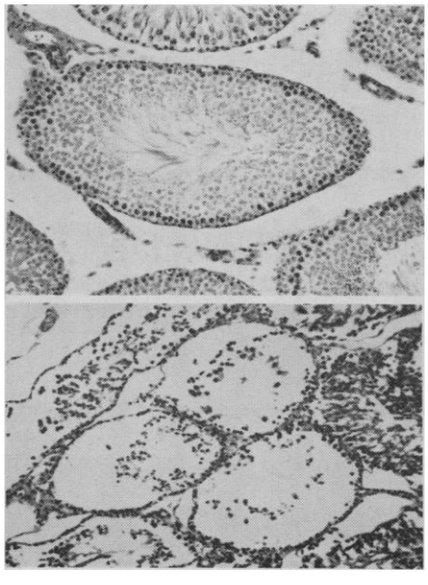

Fig. 2. (Top) Normal seminiferous tubules of a rat. (Bottom) Changes in the seminiferous tubules of a rat treated with $NaHCO_3$ while breathing 95- to 98-percent O_2 for 30 days.

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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-