concepts. We still strongly believe that the H and M types of LDH are geared for functioning in aerobic and anaerobic environments, respectively. To completely prove this point of view, it is important to demonstrate the existence of ternary complexes of the H-type LDH in vivo.

JOHANNES EVERSE
ROBERT L. BERGER
NATHAN O. KAPLAN
Department of Chemistry, University of California, San Diego, La Jolla 92037 and *Laboratory of Technical Development, National Heart and Lung Institute, Bethesda, Maryland 20014*

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Production of Urinary Bladder Carcinomas in Mice by Sodium Saccharin

Abstract. *Pellets weighing 20 to 24 milligrams and containing 20 percent sodium saccharin suspended in cholesterol were surgically implanted into the urinary bladder lumens of female Swiss mice (60 to 90 days old) under ether anesthesia. Incidences of mouse bladder carcinomas in animals exposed to these pellets were 47 and 52 percent as compared with incidences of 13 and 12 percent in control mice exposed to pellets of pure cholesterol. The exposure of the mouse bladder to saccharin was very brief, because the time required for 50 percent of the compound to be eluted from the pellets was about 5.5 hours.*

Since the discovery of saccharin (2,3-dihydro-3-oxobenzisosulfonazole) in 1879 (*1*), this chemical has been utilized as a noncaloric sweetening agent in nearly all countries of the world. During the first 6 decades of the 20th century, the use of saccharin in the United States was generally limited to persons with specific medical indications. With the advent of formulations of mixtures of cyclamate and saccharin, increasingly widespread popularity and distribution of a variety of commercial beverages and foods containing these substances occurred (*2*). Thus in 1967, it was estimated (*2*) that nearly 75 percent of the population of the United States consumed some of these nonnutritive sweeteners.

Few studies of the potential carcinogenic activity of saccharin have been conducted in animals (*2*). In one long-term trial of feeding saccharin to rats, it was reported (*3*) in 1951 that "No pathological effect whatever could be attributed to saccharin at levels of 1.0 percent or less. At 5 percent only one effect was noted, in the latter part of the experiment, namely an increased incidence of the ordinarily uncommon condition of abdominal lymphosarcoma. In the 5 percent group, there were seven animals with lymphosarcomas; this number is not out of line with the incidence in comparable groups of rats, but the fact that in four of the seven rats abdominal as well as thoracic lymphosarcomas were present is unusual, since ordinarily the ratio is about 1 to 15-20. Three of these four combinations occurred in animals on experiment one hundred and two or more weeks." No indication that the urinary bladders of these rats were inspected grossly or microscopically was made (*3*). In 1957, Allen *et al.* (*4*), employing the mouse bladder pellet implantation technique in a single experiment involving an evaluation of 13 mice, observed that "Pellets containing saccharin induced a significant incidence of bladder tumours." The mouse bladder carcinogenicity of sodium cyclamate was demonstrated and assayed by the pellet implantation technique (*5*). Oral administration of a mixture of sodium cyclamate and sodium saccharin (10 : 1) at a dose of 2500 mg/day to male and female FDRL Wistar strain rats for a maximum period of 104 weeks was followed by the formation of papillary transitional cell tumors of the urinary bladder in seven males and one female; four to eight of the tumors were diagnosed as carcinomas (*6*). The decisions recently made by public health authorities in several countries of the world, including the United States, Japan, England, Canada, and Sweden, to restrict the consumption of cyclamate, but not restrict the distribution of saccharin, were apparently made (*7*) on the basis of these data (*5, 6*). The observations of Allen *et al.* (*4*) concerning saccharin were apparently not considered because so few animals were evaluated, histopathologic confirmation of diagnoses was absent, and, to our knowledge, this experiment was never repeated. In an attempt to remedy the criticisms of the study of Allen *et al.* (*4*) and to obtain a precise assessment of the mouse bladder carcinogenicity of saccharin, it was retested by the pellet implantation technique.

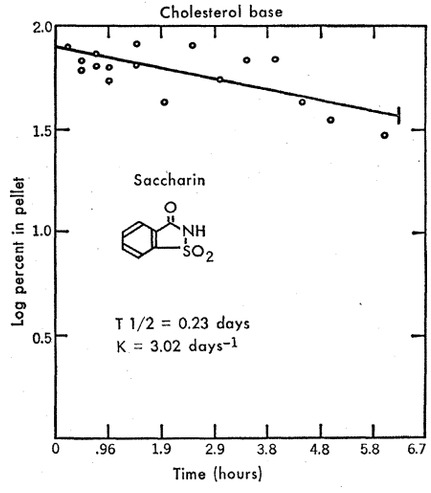


Fig. 1. Elution curve of sodium saccharin from cholesterol.

The experimental details of our use of the above-mentioned technique were reported (*5, 8*). Cholesterol, purified by recrystallization before use, and sodium saccharin (*9*) were ground separately to a fine powder in an agate mortar. The saccharin was mixed carefully with four times its weight of cholesterol before the mixture was compressed into spheroidal pellets, 5/32 inch (0.4 cm) in diameter and 20 to 24 mg in weight, with a standard face die (*5, 8*). The saccharin content of prepared pellets was measured by ultraviolet spectrophotometry (*10*) before use to assure that the quantity of saccharin had not been altered. Prepared pellets were surgically implanted into the urinary bladder lumens of female Swiss mice, 60 to 90 days old (*5, 8*). To estimate the probable exposure of the urinary bladder to saccharin, pellets were removed from some

mice at various times after implantation and the quantity of saccharin remaining in these pellets was measured (*10*). These data were used to evaluate the rate of disappearance of saccharin from the cholesterol pellets [the elution rate constant (K)] and the time required for 50 percent of the saccharin to disappear from the pellets [the 50 percent elution time (T ½)] and afforded an evaluation of the probable exposure of the bladder to the test compound (*5, 8, 10*). Pellets of identical shape and mass were made from purified cholesterol and surgically implanted in the bladder lumens of other groups of mice used as controls.

The following procedure was used for the carcinogenicity experiments (*5, 8*). Pellets composed of either pure cholesterol or sodium saccharin and cholesterol (1 : 4) were surgically placed in the bladder lumens of duplicate groups of 100 mice (*5, 8*). The animals in each group were allowed to survive 13 months, the survivors were killed, and the bladders of animals surviving more than 175 days were grossly and microscopically inspected. Bladder carcinomas were evaluated by the criteria (*11*) that were used to evaluate bladder carcinomas produced in mice by sodium cyclamate (*5*). Carcinomas that infiltrated into the subepithelial connective tissue were classified as stage I; those which additionally infiltrated into the muscular layers of the bladder as stage II; and those which additionally penetrated through the serosal layer of the bladder or presented local pelvic metastases as stage III. The total incidence of carcinomas was used to assess carcinogenicity, and statistical comparison by the exact method for 2 by 2 tables of the incidence of carcinomas related to the introduction of pellets composed of saccharin and cholesterol was made with that observed in mice exposed to pellets of cholesterol only (*12*). All organs except the brain were inspected grossly, and histologic examination of representative tissues, stained with hematoxylin and eosin, was made. This experiment was conducted at the same time as that reported previously (*5*) with sodium cyclamate, and thus the control groups given cholesterol alone were the same for both experiments.

The calculated time of exposure of the mouse bladders to sodium saccharin, the elution rate constant, and the 50-percent elution time are presented in Fig. 1. Within 5.5 hours, 50 percent of the saccharin had disappeared, and by 1.5 days, 99 percent of the saccharin

Table 1. Survival of mice living more than 175 days after bladder implantation and incidence of changes in mouse bladders with implants of sodium saccharin suspended in cholesterol.

Experiment No.	No. of mice that died or were killed (days)				Average survival (days)	Squamous metaplasia	Carcinomas					
	175–250	251–325	326–400	Total			Stages I	Stages II	Stages III	Total	Percentage	P value
Cholesterol alone												
1	1	3	59	63	378	1	7	1	0	8	13	
2	0	4	39	43	394	3	4	1	0	5	12	
Saccharin + cholesterol (20 : 80)												
1	1	3	62	66	375	3	26	4	1	31	47	$<<.001$
2	0	1	63	64	396	6	25	6	2	33	52	$<<.001$

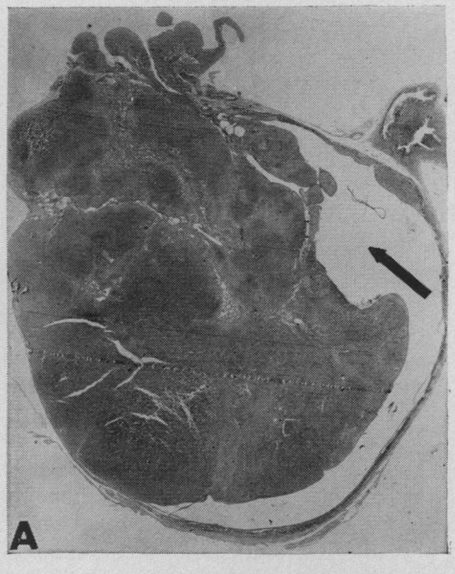
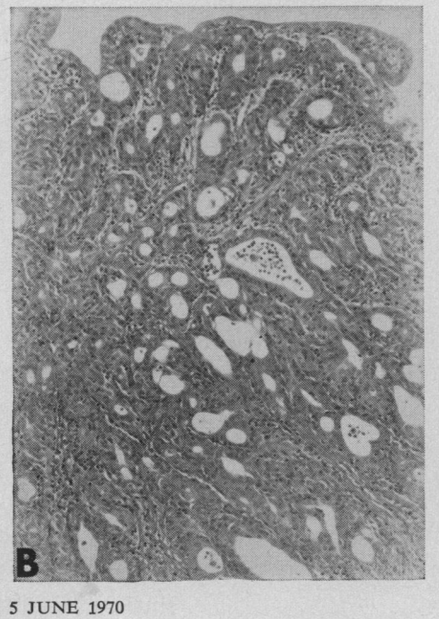
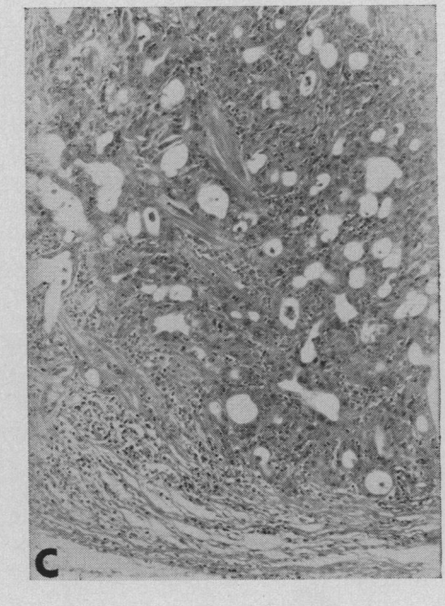
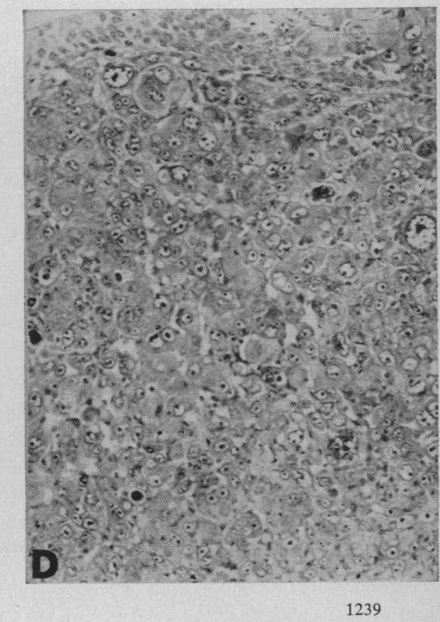


Fig. 2. Histologic appearance of mouse urinary bladder carcinomas induced by sodium saccharin. (A) Large bladder carcinoma nearly occluding lumen; note space occupied by pellet (arrow), stage III. Hematoxylin and eosin (× 5.7). (B) Invasive transitional cell carcinoma; note squamous and glandular metaplasia. Hematoxylin and eosin (× 53). (C) Invasive bladder carcinoma demonstrating complete muscular and serosal penetration, stage III. Hematoxylin and eosin (× 89). (D) High power magnification of (A) demonstrating high degree of mitotic activity, pleomorphism, and multinuclear and multinucleolar cells. Hematoxylin and eosin (× 177).







had been eluted from the pellets. This rapid exposure of the bladder to saccharin is comparable to that observed for sodium cyclamate (*5*) and the 8-methyl ether of xanthurenic acid (*13*), compounds demonstrated to possess significant carcinogenic activity for the mouse bladder when suspended in cholesterol and tested by the pellet implantation technique (*5*, *8*).

The survival of mice for more than 175 days after surgery, the average length of survival, and the incidences of squamous metaplasia and carcinoma in the bladders were compared in the duplicate test and control groups of mice (Table 1). The first bladder carcinoma was observed in a mouse treated with saccharin that died 293 days after surgery. Statistical comparisons in both experiments demonstrated that mice exposed to sodium saccharin exhibited significantly higher incidences of bladder carcinomas (47 and 52 percent) than did the controls (13 and 12 percent). The carcinomas in mice exposed to saccharin were often visualized on gross examination, were frequently multiple within the same bladder, were more often invasive into muscle ($P = .009$), and were often composed of cells demonstrating high degrees of mitotic activity and pleomorphism (Fig. 2). Squamous and glandular metaplastic epithelial alterations were more frequent in mice exposed to saccharin. No benign papilloma was seen in the bladders of saccharin-treated or control mice. No other tissues of mice exposed to sodium saccharin demonstrated a tumor incidence significantly different from that of the control groups of mice.

The degree of histologic malignancy (Fig. 2) observed in the bladder tumors produced by saccharin was more severe than that seen in mouse bladder carcinomas produced by sodium cyclamate (*5*). Moreover, the bladder carcinomas produced by saccharin were as malignant in appearance as those induced in mice, rats, and dogs (see *14*) by feeding the potent urinary bladder carcinogen *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide, and as severe as those obtained by testing *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (*15*) or other carcinogens (*8*, *13*) by the mouse bladder pellet implantation technique.

GEORGE T. BRYAN
ERDOĞAN ERTÜRK, OSAMU YOSHIDA
Division of Clinical Oncology, University of Wisconsin Medical School, Madison 53706

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Testing the Vision of Cataract Patients by Means of Laser-Generated Interference Fringes

Abstract. *In a new technique for measuring the visual acuity of cataract patients the light from a laser is used to form interference patterns of variable fineness on the patient's retina. The fineness of the interference pattern that the patient can detect gives an indication of the potential for improved vision. Comparison of this estimate of the potential with the patient's vision after cataract extraction shows that this test can indicate the condition of the fovea behind a cataract.*

A cataract, which is a loss of lens transparency, most often occurs in persons past middle age and appears to be part of the general aging process. In addition, cataracts can occur secondary to ocular or systemic disease and from traumatic or perforating injury to the globe. A developing cataract causes a gradual and painless loss of vision. When the cataract has progressed to the point where the reduction in vision remaining after the correction of refractive error interferes with normal activities, the only treatment is surgical removal of the lens. Good vision may be surgically restored if changes in the fovea have not occurred prior to surgery. The lens opacities often make it difficult to detect macular changes by ophthalmoscopic examination. The usual methods of preoperative testing such as discrimination between two point images and color perception may be poor indicators of the functional integrity of the fovea centralis.

Campbell and Green (*1*) have produced high-luminance interference fringes on the retina with a low-power gas laser. These fringes are not degraded by ordinary optical aberrations. If one views the fringes with a piece of tissue paper or milk glass held over the eye, one sees the fringe pattern disturbed (see cover). However, it is still possible to resolve fine stripes corresponding to the equivalent of 20/20 visual acuity because microscopic holes in these materials allow portions of the focused beams to pass unscattered. If an interferometric technique could produce fine, high-contrast fringes on the retina behind a cataractous lens, it would make it possible to determine the condition of the fovea. This report presents the results of a series of experiments on patients with cataracts to determine the feasibility of preoperatively testing the visual potential by means of laser-generated interference fringes.

The method used to produce interference fringes on the retina will be briefly described here; the full details are available elsewhere (*1*, *2*). The output from a low-power, helium-neon gas laser was optically divided into two parts. A lens projected an image of the doubled laser source into the eye of the patient. This can be done with safety