(Fig. 2) and the loss of inhibition achieved after treatment of ribosomes with Sepharose linked ribonuclease.

The radioimmunoassay procedure is capable of detecting ribosomal antibodies with considerably greater sensitivity than previously utilized assays. The specificity of the antibody appears to be similar, that is, related partially to RNA, although the relationship of ribosomal antibodies to nuclear ribonucleoprotein and soluble cytoplasmic ribonucleoprotein antibodies remains to be clarified.

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References and Notes

1. P. H. Schur, L. A. Moroz, H. G. Kunkel, *Immunochemistry* **4**, 447 (1967); B. C. Sturgill and M. R. Preble, *Arthritis Rheum.* **10**, 538 (1967).
2. B. C. Sturgill and R. R. Carpenter, *Arthritis Rheum.* **8**, 213 (1965).
3. J. C. Homberg, M. Rizzetto, D. Doniach, *Clin. Exp. Immunol.* **17**, 617 (1974); F. B. Bianchi, M. Rizzetto, P. Penfold, G. T. Swana, D. Doniach, *ibid.*, p. 629.
4. M. Mattioli and M. Reichlin, *Arthritis Rheum.* **17**, 421 (1974).
5. S. Penman, *Science* **154**, 786 (1966).
6. I. Faiferman, A. O. Pogo, J. Schwartz, M. E. Kaighn, *Biochim. Biophys. Acta* **312**, 492 (1973).
7. I. Faiferman, M. G. Hamilton, A. O. Pogo, *ibid.* **204**, 550 (1970).
8. J. S. Roth, *J. Biol. Chem.* **231**, 1085 (1958).
9. A. S. Cohen, W. E. Reynolds, E. C. Franklin, J. P. Kulka, M. W. Ropes, L. E. Shulman, S. L. Wallace, *Bull. Rheum. Dis.* **21**, 643 (1971).
10. We thank J. Winfield for supplying serums from the University of Virginia Hospital and L. Legrand for technical assistance. Supported by grant AM 13721 from the Public Health Service and by the Lupus Foundation.
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24 March 1977; revised 2 May 1977

13-*cis*-Retinoic Acid: Inhibition of Bladder Carcinogenesis Induced in Rats by *N*-Butyl-*N*-(4-hydroxybutyl)nitrosamine

Abstract. *Transitional cell carcinoma was induced in the bladders of male Fischer rats by 12 oral doses of the carcinogen* N-*butyl*-N-*(4-hydroxybutyl)nitrosamine. Feeding of 13-*cis*-*retinoic acid after completion of carcinogen treatment diminished the number and severity of cancers and other proliferative lesions of the bladder.*

In a previous study we demonstrated that 13-*cis*-retinoic acid can inhibit the development of bladder cancer induced by *N*-methyl-*N*-nitrosourea in female Wistar/Lewis rats (*1*). A high percentage of these malignancies were squamous cell carcinomas (30 to 40 percent), while in humans squamous bladder cancers account for much less of the total (*2*). We therefore wished to verify our results in another experimental system in which carcinogenesis produces almost entirely transitional cell tumors (*3*). In the study reported here, bladder cancer was induced in Fischer male rats with 12 intragastric doses of 200 mg of *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (OH-BBN) (*4*) over a period of 6 weeks (Table 1). One week following the final dose, 21 of the 43 treated rats were placed on diets containing 240 mg of 13-*cis*-retinoic acid per kilogram of diet. The animals were killed 6 months after the initial carcinogen treatment. The bladders were inflated with 10 percent formalin, and after fixation were transected with two cuts in a dorsoventral plane to yield an anterior and a posterior dome-shaped area and a median cylindrical area. Each of the three areas was serially sectioned (5 μm) in the plane of the original cuts, and stained with hematoxylin and eosin. The slides were randomized, coded, and evaluated by three of us (G.M.F., D.G.G., and S.F.S.) using two similar semiquantitative scoring systems. One system has been described previously (*5*), and the other will be described at length in a later report. There was excellent agreement between all three pathologists, and the results were therefore averaged.

The transitional cell carcinomas and other proliferative epithelial lesions were ranked according to their relative grade or atypia score (Table 1). These were determined, in part, by the degree of epithelial differentiation and of cellular anaplasia and by the number of mitotic figures. The results indicate that 13-*cis*-retinoic acid inhibited the development of transitional cell carcinomas in the bladder. Significantly fewer animals fed this retinoid had severe (high-grade) cancers. The same was true for the other proliferative epithelial lesions; fewer animals fed

Table 1. Inhibition of bladder carcinogenesis by 13-*cis*-retinoic acid. Beginning at 6 weeks of age, male Fischer rats were dosed twice weekly, intragastrically, each time with 200 mg of OH-BBN (*4*), dissolved in 0.5 ml of 20 percent ethanol. A total of 2400 mg was given over a period of 6 weeks. One week after the final dose, 21 of the rats were placed on a diet containing 240 mg of 13-*cis*-retinoic acid (Hoffmann-La Roche) per kilogram of diet. The retinoid was obtained as a gelatinized beadlet preparation and blended into a powdered diet (Wayne Lab Meal). The control group was fed the same diet containing gelatinized beadlet material without retinoid. Animals were killed 6 months after the initial carcinogen treatment. Histological sections from the anterior, median, and posterior areas of each bladder were randomized, coded, and evaluated by three pathologists using similar semiquantitative scoring systems, and the results were averaged. Values for *P* were derived from chi-square and Student *t*-tests. The diagnosis of transitional cell carcinoma was based on the presence of invasion of underlying connective tissue or invasion of smooth muscle, and of moderate to marked histologic or cytologic atypia. Atypia was scored on a scale from 0 to 5 depending on the presence of increased cellularity, hyperchromasia, prominent nucleoli, pleomorphism of cellular and nuclear size and shape, loss of cell polarity and orientation, and presence of mitoses. Low-grade cancers had atypia scores below 3; high-grade, 3 and above. Other proliferative epithelial lesions included noninvasive papillomas and discrete hyperplastic lesions that did not meet the diagnostic criteria necessary to be classified as carcinomas. Lesions were considered to have atypical epithelia if atypia scores were 2 or above. An atypia score of 1 was considered minimal; a score of 2, moderate; and a score above 2, severe. Criteria for scoring atypia have been described in detail (*5*). In addition to the rats described here, 25 rats, which received no carcinogen, were fed 13-*cis*-retinoic acid. Bladders from these animals showed no histopathological abnormalities. Bladders from 23 rats, which received neither carcinogen nor retinoid for the duration of the experiment, were also histologically normal.

Treatment	Rats (No.)	Rats with transitional cell carcinoma (No.)		Rats with other proliferative epithelial lesions (No.)		Total proliferative epithelial lesions, including cancers (No.)	Rats with epithelial atypia (No.)			Mean atypia score
		Low-grade	High-grade	Typical epithelium	Atypical epithelium		Minimal	Moderate	Severe	
OH-BBN alone	22	1	9	4	9	47	5	6	11	2.0
OH-BBN and 13-*cis*-retinoic acid	21	3	4	7	3	24	10	6	5	1.1
P			<.05		<.01	<.01	<.025		<.025	<.01

13-*cis*-retinoic acid had such lesions with accompanying epithelial atypia. Moreover, the total number of proliferative lesions, including cancers, was lower by one-half in the animals fed 13-*cis*-retinoic acid. Finally, feeding of the retinoid also diminished the severity of atypical cellular changes throughout the epithelium. Although some epithelial atypia was found in all animals treated with OH-BBN, there were significantly fewer animals with severe atypia in the group fed 13-*cis*-retinoic acid after the treatment with OH-BBN.

This study confirms our earlier findings that 13-*cis*-retinoic acid can inhibit both the incidence and the severity of neoplastic alterations induced in the bladders of rats with carcinogens. The animals were not started on the diet containing the 13-*cis*-retinoic acid until after the carcinogen exposures, which precludes the possibility that the inhibition was exerted during the initiation phase of carcinogenesis. As in our previous study, it is also unlikely that the inhibition was due to a generalized toxic effect, as no signs of retinoid toxicity (*6*) were observed.

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References and Notes

1. M. B. Sporn, R. A. Squire, C. C. Brown, J. M. Smith, M. L. Wenk, S. Springer, *Science* **195**, 487 (1977).
2. F. K. Mostofi and J. E. Leestma, in *Pathology*, W. A. D. Anderson, Ed. (Mosby, St. Louis, 1971), p. 829; G. R. Prout, in *Cancer Medicine*, J. F. Holland and E. Frei, Eds. (Lea & Febiger, Philadelphia, 1973), p. 1670.
3. S. Fukushima, M. Hirose, H. Tsuda, T. Shirai, K. Hirao, M. Arai, N. Ito, *Gann* **67**, 81 (1976); E. Kunze, A. Schauer, S. Schatt, *Z. Krebsforsch.* **87**, 139 (1976).
4. Synthesized by E. Reist, Stanford Research Institute, Menlo Park, Calif., by the method of D. Schmähl and F. W. Krüger [*Arzneim. Forsch.* **22**, 999 (1972)].
5. R. A. Squire, M. B. Sporn, C. C. Brown, J. M. Smith, M. L. Wenk, S. Springer, *Cancer Res.* **37**, 2930 (1977).
6. T. Moore, in *The Vitamins*, W. H. Sebrell and R. S. Harris, Eds. (Academic Press, New York, ed. 2, 1967), vol. 1, pp. 280–294.
7. We thank Hoffmann-La Roche Inc., Nutley, N.J., for 13-*cis*-retinoic acid, L. Keefer and M. Litwack for assistance in obtaining OH-BBN, and M. O'Boyle and D. Little for help with the manuscript.

1 August 1977

Sex-Ratio Adjustment in the Common Grackle

Abstract. *From the nestling period through maturity, female grackles are distinctly smaller than males and presumably cost less to rear. Individual birds nesting early in the season lay more female eggs than those nesting later, and in large broods, mortality after hatching consistently favors female fledglings. The first result suggests an adaptive nonrandom meiosis that anticipates seasonal conditions of food availability; the second implies a brood reduction strategy consistent with Fisher's prediction that differential mortality in sexually dimorphic species should favor the less expensive sex.*

Fisher (*1*) hypothesized that natural selection will favor equal parental expenditure on each sex until the end of parental care. If individual males cost as much to rear as individual females, selection should result in population sex ratios of unity at the end of parental care. If one sex costs less to raise than the other, a numerical excess of that sex would be expected at independence. Unless extreme inbreeding occurs (*2*), the hypothesis should apply to all diploid organisms in which reproductive investment in one offspring diminishes parental ability to invest in another.

Despite its generality, Fisher's hypothesis has not been adequately tested with birds. This is surprising because females contribute the sex-determining chromosome in this group (*3*). Nonrandom deviations in primary sex ratio must result from maternal control and cannot reflect conflicting maternal and paternal influences. In principle it is possible to compare initial with subsequent sex ratios produced by heterogametic females, thereby separating the mechanisms of segregation distortion from differential mortality. Dual maternal and paternal influences obscure such comparisons in mammals and other organisms in which males contribute the sex-determining chromosome (*4*). I now report that adjustment of both primary and subsequent sex ratios occurs in a species of bird and interpret these results in an evolutionary context.

Sex-ratio control consistent with Fisher's hypothesis may come about through parental manipulation of either primary or later sex ratios. The least interesting case concerns organisms in which it costs as much to raise one sex as the other. In such species, the primary population sex ratio should be unity and should be maintained until the end of parental care. More interesting are organisms among which one sex costs more for the parents to raise than the other, as might occur in species with strong sexual size dimorphism. The simplest situation is a hypothetical population for which future conditions of parental care are the same for all adults about to breed: the population sex ratio should favor the less expensive sex from conception through independence. A more likely circumstance is that different breeding adults face different conditions of parental care, so that some are more capable of rearing the "expensive" sex than others. Proximate factors might be differences in physical condition, weather, or assistance from mates. If individuals can predict the conditions under which they will later care for young, primary sex ratios should anticipate their future ability to invest. Such anticipation may occur when females know their physical condition relative to others in the population (*5*) or, more likely, when individuals breeding early in a highly seasonal environment consistently face circumstances different from those breeding later. If parents cannot predict future conditions when they commence breeding, the sex ratio after conception may be adjusted by selectively resorbing embryos or by killing the young in a manner adaptive for the parents (*1*, *5*). Such a method might be expected in a bird in which an adaptive pattern of brood reduction permits parents to cope with seasonal conditions that frequently deteriorate during breeding attempts (*6*, *7*).

Table 1. Embryonic sex ratios among common grackles from two seasons at Dexter, Michigan.

Clutch size	Nests (No.)	Males (No.)	Females (No.)
3	4	4	8
4	25	51	43
5	63	151	143
6	4	5	5
Total	96	211	199

Common grackles (*Quiscalus quiscula*, Icteridae) are likely candidates for studies of sex-ratio adjustment. Female fledglings weigh 82 percent as much as males of a similar age, a dimorphism maintained through maturity (*8*). Thus, one expects a sex ratio at the end of parental care that favors female young requiring less food than males. Preliminary evidence indicates that fledgling males of female size suffer disproportionate mor-