Preventive and Enhancing Effects of Retinoids on the Development of Naturally Occurring Tumors of Skin, Prostate Gland, and Endocrine Pancreas in Aged Male ACI/segHapBR Rats [1,2]

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ABSTRACT—The effects of dietary retinoids on the development of naturally occurring tumors in retired breeder male ACI/segHapBR rats were investigated. Groups of rats (21–25 mo of age, an age when early neoplasms first appear and tumor incidences are generally low) were fed diets containing 1 of 3 retinoids—*all-trans-N*-4-(4-hydroxyphenyl)retinamide (4-HPR), 783 mg/kg diet; *all-trans*-N-(4-pivaloyloxyphenyl)retinamide (4-PPR), 951 mg/kg; or *all-trans*-4-*N*-(2-hydroxyethyl)retinamide (2-HER), 687 mg/kg—or control diet for up to 54 weeks (average, 33 wk). Rats were maintained until less than 20% remained and the experiment was terminated. Contributing causes of death were determined, and a complete necropsy was performed for each rat. There was no difference between the retinoid-treated rats and control rats in the average age at death (30–31 mo) or in the average experimental survival time (29–35 wk), in the proportions of tumor-bearing rats (95.6–100%), or in the average number of organs with tumor per rat (2.1–2.5). The incidences of pancreatic islet cell adenoma and skin tumors were significantly different between control and some retinoid-treated groups. 4-PPR and 2-HER significantly enhanced pancreatic islet cell adenoma yields ($P<.025$ and 0.05, respectively) whereas 4-HPR significantly inhibited epithelial and connective tissue skin tumor yields ($P<.025$). Incidences of skin and prostate tumors were lower than in controls, but not significantly, in rats receiving 4-PPR and 2-HER. Most of the islet cell adenomas were shown, by avidin-biotin-peroxidase complex immunocytochemistry, to be insulinomas. 4-HPR would seem to be the most effective retinoid in the group, inasmuch as it prevented skin tumor development, may have slightly decreased the incidence of prostate tumors, and did not enhance islet cell tumor incidence.—JNCI 1985; 74:517–524.

Natural and synthetic analogues of vitamin A (retinoids) are important substances for control of both cellular differentiation and cellular proliferation in epithelial tissues (*1–4*). Vitamin A also has many important physiologic functions in normal growth, vision, and fertility (*2, 5*). Retinoids were shown to have preventive effects on the development and progression of preneoplastic lesions, such as hyperplasia and metaplasia, both in in vivo and in vitro experimental systems (*2, 6, 7*), as well as on neoplastic lesions in skin and viscera. Most in vivo experimental studies on the effects of retinoids have been performed with chemically induced tumors (*6*). Only two reports (*8, 9*) deal with the influences of retinoids on the induction of spontaneous tumors.

ACI rats develop naturally occurring tumors in various organs, including the endocrine organs, testis, prostate gland, and skin, at high incidences with advancing age (*10, 11*). Studies have demonstrated that retinoids can inhibit and reverse the hyperplastic and metaplastic changes induced by chemical carcinogens (*1, 12–14*) and testosterone (*15*) in mouse prostate organ cultures. There have been no reports, however, on the effects of retinoids on in vivo prostate carcinogenesis. Some epidemiologic studies have suggested a possible enhancing effect of retinoids on human prostate cancer (*16*). Both virgin and exbreeder male ACI rats are prone to the spontaneous development of intraalveolar atypical hyperplasias and invasive carcinomas (35%) of the prostate gland by 33 months of age (*17*). In this paper, we evaluated the possible anticarcinogenic effect on spontaneous prostate lesions and on other spontaneous tumors by 3 retinoids in aging male ACI rats.

MATERIALS AND METHODS

Animals.—A total of 195 aged male ACI/segHapBR rats (retired breeders) were procured from Harlan Sprague-Dawley, Inc. (Cumberland, IN). The ages of these animals were from 20 to 24 months at the time of receipt. In our laboratory, all animals were housed by birth date, 3 rats/13×15-inch polycarbonate cage, on heat-treated hardwood chips, with water and Purina Laboratory Chow (block form; Ralston Purina Co., St. Louis, MO) available ad libitum. All cages and water bottles were changed and sanitized twice per week. Animals were checked twice daily for death or illness and

ABBREVIATIONS USED: ABC=avidin-biotin-peroxidase complex; 2-HER=*all-trans*-4-*N*-(2-hydroxyethyl)retinamide; 4=HPR=*all-trans-N*-4-(4-hydroxyphenyl)retinamide; 4-PPR=*all-trans-N*-(4-pivaloyloxyphenyl) retinamide.

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were weighed every 2 weeks. All animals were held under consistent animal-room conditions of 72±4°F, 50±20% relative humidity, and a 12-hour light-12-hour dark cycle. No skin or other tumors were evident clinically at initiation of the experiment.

Retinoid treatment.—4-HPR, 4-PPR, and 2-HER were synthesized by Dr. Y. Fulmer Shealy at Southern Research Institute (Birmingham, AL), under a U.S. Public Health Service contract. Their purities were more than 99.5% as determined by high-performance liquid chromatography. Prior to test-diet administration, 4 experimental groups were constructed by random distribution of the surviving rats from each birth date-defined cohort of animals. This resulted in the formation of 3 groups containing 45 animals (groups 1, 2, and 3) and 1 group of 44 animals (group 4). All surviving animals in each group were transferred to their respective test diets when the animals ranged between 21 and 25 months of age. Each retinoid was added to the appropriate diet at 2 mmol/kg diet. Diets contained 783 mg 4-HPR/kg (group 2), 951 mg 4-PPR/kg (group 3), or 687 mg 2-HER/kg (group 4) and were prepared with Purina Laboratory Chow (meal form) as follows: 3.92 g 4-HPR, 4.76 g 4-PPR, or 3.44 g 2-HER were dissolved in a common vehicle solution containing 62.5 ml ethanol, 187.5 ml Trioctanoin (Fluka, Hauppauge, NY), 2.5 mg Tenox 20 (Eastman Chemical Products, Inc., Kingsport, TN), and 2.5 ml DL-tocopherol (Sigma Chemical Co., St. Louis, MO) and then added slowly to 5-kg batches of diet during blending. All diets were prepared in 5-kg batches and mixed on a Hobart mixer for 30 minutes. Control diet (group 1) was prepared identically to test diets but with omission of retinoids. Diets were stored in opaque airtight color-coded pails and were consumed within 1 week of mixing. The experimental period lasted for a maximum of 54 weeks following the placement of animals on test diets. At that time (terminal sacrifice), all remaining rats (13–20% by group) were killed and necropsied.

Pathology examination.—All animals found dead or sacrificed received a thorough and complete necropsy and were studied macroscopically for tumors. The weights of the prostate gland, testis, and adrenal, thyroid, and pituitary glands were determined. For each rat the contributing cause(s) of death was determined (*18*). All organs were fixed in 10% Formalin, and sections were stained with hematoxylin and eosin. For immunocytochemistry of the pancreatic islet cell lesions, Formalin-fixed sections of pancreas were stained by the ABC immunocytochemistry technique (*19*) with the use of the Vectastain ABC kit (Vector Laboratories, Inc., Burlingame, CA). Rabbit antibodies to human somatostatin or glucagon were obtained from DAKO Corp. (Santa Barbara, CA), and guinea pig antibodies to bovine insulin were obtained from Miles Laboratories, Inc. (Elkhart, IN). Dilutions of 1:250 to 1:1,000 were used for staining.

Statistics.—Data were evaluated by the Student's *t*-test for body and organ weights, age, survival, and number of organs with tumor per rat or by chi-square analysis with Yates' correction for comparisons of tumor incidence.

RESULTS

Survival Time, Body Weight, and Organ Weights

Table 1 summarizes mean experimental survival times, mean age at start of experiment and at death, and initial and final body weights. There were no significant differences in these data for body-weight gain or loss among the groups. Generally, the mean body weights of all groups decreased as the rats aged. Mean organ weights were not significantly different among the groups (data not given).

Incidence of Tumors and Cause of Death

The incidences of tumor-bearing rats and number of organs with tumor per rat are shown in table 1. Most rats (95.6–100%) in all groups had tumors that appeared in as many as 6 different organ sites. The contributing causes of death in each group are summarized in table 2. The major causes of death were neoplasms in all groups. There were no differences in the causes of death associated with the administration of retinoids.

Skin, Subcutaneous Tissue, and Genitourinary Organs

Histopathologic findings and incidences of tumors in skin, subcutaneous tissue, and genitourinary organs are given in table 3. The incidences of tumors of skin and subcutaneous tissues in the retinoid-treated groups were

TABLE 1.—*Mean survival time, body weight, and tumor incidence: Aged male ACI/segHapBR rats*

Group	No. of rats	Treatment	Age[a], mo		Survival: No. of wk on test[a]	Body weight[a], g		No. (%) of tumor-bearing rats	No. of organs with tumor per rat[a]
			At start of expt	At death		At start of expt	Final		
1	45	None (controls)	23±1	30±4	29±17	376±33	302±47	43 (95.7)	2.3±1.3
2	45	4-HPR	23±1	30±6	32±16	389±32	303±33	45 (100)	2.1±1.1
3	45	4-PPR	23±2	31±4	35±17	388±35	299±39	44 (97.8)	2.4±1.3
4	44	2-HER	23±1	31±3	35±16	397±29	298±31	44 (100)	2.5±1.3

[a] Mean ± SD.

TABLE 2.—*Contributing causes of death: Aged male ACI/segHapBR rats*

Contributing cause of death	No. (%) of rats with lesions in groups:[a]			
	1	2	3	4
Neoplasms	31 (68.9)	31 (68.9)	28 (62.2)	30 (68.2)
Pituitary gland	15	13	10	11
Leukemia	5	1	4	2
Adrenal gland	1	5	3	3
Skin	2	2	3	2
Subcutaneous tissue	4	1	2	3
Stomach	0	2	2	1
Peripheral nerve	0	1	2	1
Intestine	0	1	0	1
Miscellaneous[b]	4	5	2	6
Nonneoplastic lesions	8 (17.8)	6 (13.3)	10 (22.2)	5 (11.4)
Nephropathy	5	0	3	2
Others[c]	3	6	7	3
Terminal sacrifice	6 (13.3)	8 (17.8)	7 (15.6)	9 (20.5)

[a] Group 1: controls (45 rats); group 2: 4-HPR treated (45 rats); group 3: 4-PPR treated (45 rats); group 4: 2-HER treated (44 rats).
[b] Miscellaneous tumors included 1–2 tumors from each of the following tissues: tongue, thyroid gland, thymus gland, kidney, mammary gland, salivary gland, pharynx, lung, and prostate gland.
[c] Others included infectious diseases, lung congestion, lung hemorrhage, and unknown.

generally lower than in the control group, and the difference between group 1 (control) and group 2 (4-HPR) was statistically significant ($P<.025$), especially for epithelial tumors. A few rats had both epithelial and connective tissue tumors. Testicular interstitial cell tumors appeared to be the most frequent genital tumors in rats of each group. The incidences of prostate tumors in group 2 (4-HPR) and in group 4 (2-HER) were lower than in group 1 (controls), but these differences were not statistically significant. The prostate tumors were similar morphologically to those previously reported in aging ACI rats (*17*). The incidences of atypical intraalveolar prostate hyperplasias were 28/44 (63.6%), 24/40 (60%), 27/44 (61.4%), and 29/43 (67.4%) for rats in groups 1–4, respectively. There were no significant differences in incidences of hyperplasia or tumors of the genitourinary organs among the groups.

Histopathology of Tumors in Various Endocrine Organs

Histologic findings and incidences of spontaneous tumors in endocrine organs are given in table 4. The incidence of pheochromocytoma was increased, but not significantly, in rats administered the 2-HER diet (group 4) as compared to the incidence in the control group. Islet

TABLE 3.—*Tumors in skin, subcutaneous tissue, and genitourinary organs: Aged male ACI/segHapBR rats*

Organ	Histologic classification	No. of tumors (% of rats with tumors) in group:[a]			
		1	2	3	4
Skin	Keratoacanthoma	4 (8.9)	1 (2.2)	2 (4.4)	0 (–)
	Squamous cell carcinoma	0 (–)	0 (–)	2 (4.4)	1 (2.3)
	Basal cell carcinoma	1 (2.2)	0 (–)	0 (–)	1 (2.3)
	Sebaceous adenoma	1 (2.2)	0 (–)	0 (–)	0 (–)
Subcutaneous tissue	Fibrosarcoma	2 (4.4)	1 (2.2)	2 (4.4)	2 (4.5)
	Hemangiosarcoma	1 (2.2)	0 (–)	0 (–)	0 (–)
	Hemangiopericytoma	2 (4.4)	0 (–)	0 (–)	0 (–)
	Schwannoma	0 (–)	1 (2.2)	0 (–)	1 (2.3)
	Lymphoma	0 (–)	0 (–)	0 (–)	1 (2.2)
	Fibroma	1 (2.2)[b]	0 (–)	1 (2.2)	0 (–)
	Lipoma	1 (2.2)	0 (–)	0 (–)	0 (–)
Total		12/45[c] (26.7)	3/45[c] (6.7)[d]	7/45[c] (15.6)	5/44[c] (11.4)
Testis	Interstitial cell tumor	21/45[c] (46.7)	24/44[c] (54.5)	25/45[c] (55.6)	27/44[c] (61.4)
Prostate gland	Adenoma	17 (38.6)	8 (20.0)	14 (31.8)	9 (20.9)
	Carcinoma	5 (11/4)	3 (15.0)	5 (11.4)	5 (11.6)
Total		19/44[c] (43.2)	11/40[c] (27.5)	17/44[c] (38.6)	12/43[c] (27.9)
Seminal vesicle	Adenoma	0/45[c] (0)	0/45[c] (0)	0/45[c] (0)	1/44[c] (2.3)
Mammary gland	Adenoma	0 (0)	1 (2.2)	0 (0)	0 (0)
	Adenocarcinoma	0 (0)	0 (0)	1 (2.2)	0 (0)
Total		0/45[c] (0)	1/45[c] (2.2)	1/45[c] (2.2)	0/44[c] (0)
Kidney	Tubular cell tumor	1/44[c] (2.3)	1/45[c] (2.2)	1/45[c] (2.2)	0/44[c] (0)
Urinary bladder	Papilloma	0/45[c] (0)	0/45[c] (0)	2/45[c] (4.4)	2/44[c] (4.5)

[a] Group 1: controls; group 2: 4-HPR treated; group 3: 4-PPR treated; group 4: 2-HER treated.
[b] Rat also had a keratoacanthoma.
[c] No. of tumors/No. of rats examined.
[d] $P<.025$, significantly different from group 1 by χ^2-test with Yates' correction.

TABLE 4.—*Tumors in endocrine organs: Aged male ACI/segHapBR rats*

Organ	Histologic classification	No. of tumors (% of rats with tumors) in groups:[a]			
		1	2	3	4
Adrenal gland	Pheochromocytoma	13 (30.2)	18 (41.9)	13 (29.5)	22 (50.0)
	Cortical adenoma	2 (4.7)	0 (0)	0 (0)	1 (2.3)
	Cortical carcinoma	1 (2.3)	0 (0)	0 (0)	0 (0)
Total		15/43[b] (34.9)	18/43[b] (41.9)	13/44[b] (29.5)	22/44[b] (50.0)
Pancreas	Islet cell adenoma	1 (2.5)	4 (9.1)	10 (23.3)[c]	8 (18.2)[d]
	Acinar cell adenoma	0 (0)	1 (2.3)	0 (0)	0 (0)
Total		1/40[b] (2.5)	5/44[b] (11.4)	10/43[b] (23.3)[c]	8/44[b] (18.2)[d]
Pituitary gland	Adenoma	13 (33.3)	16 (38.1)	13 (29.5)	15 (36.6)
	Carcinoma	7 (17.9)	3 (7.1)	5 (11.4)	4 (9.8)
Total		20/39[b] (51.3)	19/42[b] (45.2)	18/44[b] (40.9)	19/41[b] (46.3)
Thyroid gland	Follicular cell adenoma	1 (2.3)	0 (0)	0 (0)	2 (4.7)
	C-cell carcinoma	0 (0)	1 (2.5)	2 (4.7)	0 (0)
Total		1/43[b] (2.3)	1/40[b] (2.5)	2/43[b] (4.7)	2/43[b] (4.7)
Parathyroid gland	Adenoma	2/38[b] (5.3)	2/37[b] (5.4)	1/35[b] (2.9)	3/32[b] (9.4)

[a] Group 1: controls; group 2: 4-HPR treated; group 3: 4-PPR treated; group 4: 2-HER treated.
[b] No. of tumors/No. of rats examined.
[c] $P<.025$, significantly different from group 1 by χ^2-test with Yates' correction.
[d] $P<.05$, significantly different from group 1 by χ^2-test with Yates' correction.

cell adenoma was found in only 1 rat (2.5%) of the control group but was found in 4–10 rats (9.1–23.3%) in the retinoid-treated groups. The incidences of islet cell adenoma were significantly increased in group 3 (4-PPR) and group 4 (2-HER) ($P<.025$ and 0.05, respectively) as compared to the incidence in group 1 (control).

Histochemical findings of islet cell adenomas are given in table 5 and figures 1–4. All tumors contained tumor cells with immunoreactive insulin and thus were insulinomas (figs. 1, 3). Most tumors also contained cells with other immunoreactive hormones, including glucagon (fig. 2) or somatostatin (fig. 4). Insulin was present in the majority of tumor cells. Somatostatin was found only in scattered cells in most tumors but was demonstrated in the majority of tumor cells in 3 tumors (fig. 4). In a few tumors so many cells contained insulin or somatostatin that it appeared probable that some tumor cells contained both hormones (figs. 3, 4). Glucagon only was found in a few tumor cells (fig. 2). There were no differences in incidences of tumors of pituitary, thyroid, and parathyroid glands among the groups.

TABLE 5.—*Immunocytochemical characterization of islet cell tumors: Aged male ACI/segHapBR rats*

Group	No. of islet cell tumors examined	No. of tumors with cells containing:[a]					
		Insulin		Glucagon		Somatostatin	
		++	+	++	+	++	+
Controls	1	1	0	0	1	0	1
4-HPR	4	0	4	0	2	2	0
4-PPR	10	4	6	0	5	1	5
2-HER	5	3	2	0	1	0	4
Total	20	8	12	0	9	3	10

[a] ++=many tumor cells with immunoreactive hormone; +=few tumor cells with immunoreactive hormone.

Tumors in Other Organs and Nonneoplastic Lesions

Histopathologic findings and incidences of tumors in digestive, respiratory, nervous, hematopoietic, and lymphoid systems are given in table 6. Spontaneous tumors in other organs were uncommon, and the incidences of these were not significantly different among the groups. No nonneoplastic lesions, including testicular or prostatic atrophy and nephropathy, were associated with any retinoid treatment.

DISCUSSION

In this study, we demonstrated that 2 of 3 retinoids (4-PPR and 2-HER) had a clear enhancing effect on spontaneous pancreatic endocrine tumors (islet cell), whereas 1 retinoid (4-HPR) had a clear inhibiting effect on skin tumors. In addition, 2 retinoids (4-HPR and 2-HER) may have inhibited slightly the development of spontaneous prostate tumors of ACI rats. Previous studies showed that 4-HPR reaches the skin and prostate in significant concentrations after injection or oral dosing (*20*) and is relatively nontoxic (*21*). 4-HPR also has had chemopreventive effects on induced mammary cancer in rats (*22*) and mammary organ culture (*23, 24*), some of which may be associated with hormone effects (*15, 24*).

Pancreatic islet cell tumors have been induced in rats either by the combined action of streptozotocin and nicotinamide (*25*) or by radiation exposure (*26*). In general, however, spontaneous pancreatic islet cell tumors occur in low incidence in rats (1–15%) (*27*). Ward et al. (*11*) reported that islet cell tumors were observed in 14 of 216 (6.5%) aging ACI male rats from the same commercial source as the rats in this experiment. In our experiment, rats receiving 4-PPR and 2-HER had incidences of islet cell tumors of 23.3% and 18.2%, respectively.

TABLE 6.—*Tumors in the digestive, respiratory, nervous, hematopoietic, and lymphoid systems: Aged males ACI/segHapBR rats*

Organ	Histologic classification	No. of rats with tumors in groups:[a]			
		1	2	3	4
Brain	Granular cell tumor	1	0	0	2
Peripheral nerve	Malignant schwannoma	0	1	2	1
Stomach	Malignant carcinoid	0	1	0	0
	Squamous cell carcinoma	0	1	0	0
	Adenocarcinoma	0	0	1	0
	Fibrosarcoma	0	0	0	1
	Leiomyosarcoma	0	0	1	0
	Papilloma	0	1	0	0
	Fibroma	1	0	0	0
Small intestine	Cystoadenocarcinoma	0	0	0	1
	Fibroma	0	1	0	0
Large intestine	Leiomyoma	0	0	0	1
Cecum	Fibroma	1	0	0	0
Peritoneum	Mesothelioma	0	1	0	0
Liver	Hepatocellular adenoma	2	1	1	1
	Malignant mast cell tumor	0	1	0	0
Tongue	Papilloma	0	0	1	0
Salivary gland	Adenoma	0	0	0	1
	Mixed tumor	0	0	0	1
Lung	Adenocarcinoma	1	1	0	0
Pharynx	Squamous cell carcinoma	0	0	0	1
Thymus gland	Thymoma	2	0	0	1
Lymph node	Hemangiopericytoma	0	1	0	0
Spleen	Leukemia	5	2	4	2

[a] Group 1: controls (45 rats); group 2: 4-HPR treated (45 rats); group 3: 4-PPR treated (45 rats); group 4: 2-HER treated (44 rats).

Enhanced yields of pancreatic tumors also were observed in hamsters fed high levels of retinoids following a single dose of bis(2-oxopropyl)nitrosamine (*28*). These pancreatic tumors histopathologically were ductular carcinoma or adenoma (*28*). Longnecker et al. (*29, 30*), however, reported that retinoids inhibited pancreatic acinar tumors induced by azaserine in rats. Because azaserine does not induce islet cell tumors, no effect was noted on these tumors. Therefore, the effects of retinoids on pancreatic carcinogenesis may depend on both the histologic type of pancreatic tumor and the species.

The cause of enhanced islet cell tumorigenesis is unknown. Speculation on the inhibition of endocrine carcinogenesis by retinoids has focused on the blocking of polypeptide hormone mitogenic effects (*31*). Conceivably, enhancement of carcinogenesis may occur through similar bioeffects. The immunocytochemical identification of insulin in 20 of 20 (100%) naturally occurring islet cell tumors establishes the origin of these neoplasms from beta cells in aging ACI rats. Also, most of the islet cell tumors with insulin-positive activity had some glucagon- and/or somatostatin-immunoreactive cells. Pancreatic endocrine tumors in humans frequently consist of tumor cells containing 1 or 4 hormones (*32–34*). The result of our immunocytochemical analysis of rat islet cell tumors is consistent with those results and those of induced islet cell tumors in rats. Our report is the first to include data on multiple endocrine tumor cell types in natural islet cell tumors of rats, although similar findings were reported for human tumors (*33*) and induced rat tumors (*25*).

Although there are many reports (*2, 7, 35–37*) that retinoids inhibit skin tumors induced by chemical carcinogens, other reports (*38, 39*) have shown that retinoids enhanced experimental skin carcinogenesis. Our experiment indicates that some retinoids may be inhibitors of spontaneous skin tumors of ACI rats as well. Pharmacokinetic studies have demonstrated that 4-HPR reaches skin and connective tissues after oral dosing (*20*), suggesting that it may have reached these organs for its chemopreventive effects in our study. Although few experiments have studied the possible inhibition of connective tissue tumors, at least 1 has shown the inhibition of virus-induced sarcoma (*40*). Our findings on the inhibition of epithelial and connective tissue tumors in ACI rats are supported by the findings of a similar incidence of these tumors (25%) in controls in our other aging ACI rat study (26.7% incidence in controls in the present study). Thus the incidences of 6.7 and 11.4% in two retinoid-treated groups appears even more convincing.

Reports of several studies that used in vitro systems (*12–15*) suggest that retinoids have a possible preventive effect against prostate carcinogenesis, but no study reports an inhibiting effect of retinoids on in vivo prostate tumorigenesis. We cannot show a statistically significant effect of retinoids on prostate carcinogenesis, but our results are consistent with an inhibiting effect of retinoids against prostate tumors that has been suggested by in vitro experimental systems. ACI rats generally have a low incidence of early preneoplastic and neoplastic prostate lesions at 21–25 months of age—the ages of our rats when we began our study (*17*). Thus the retinoid exposure may have occurred too late to have any preventive effect, inasmuch as early lesions presumably already were present.

Testicular atrophy has been reported in retinoid-treated rats (*41*), dogs (*42*), and hamsters (*43*). However, our experiment demonstrates no difference in the incidence of testicular atrophy in any group. This lesion may have been masked by the common occurrence of multiple testicular tumors.

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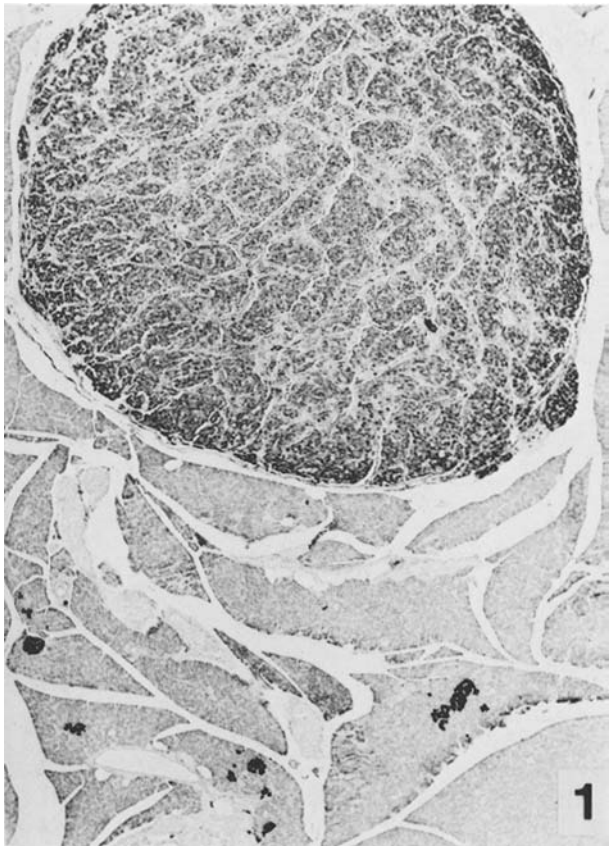


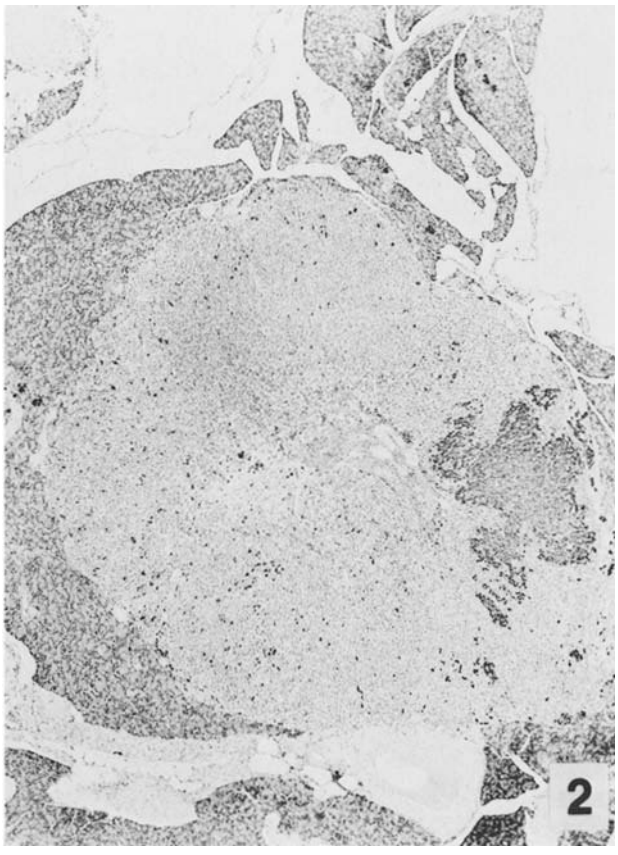
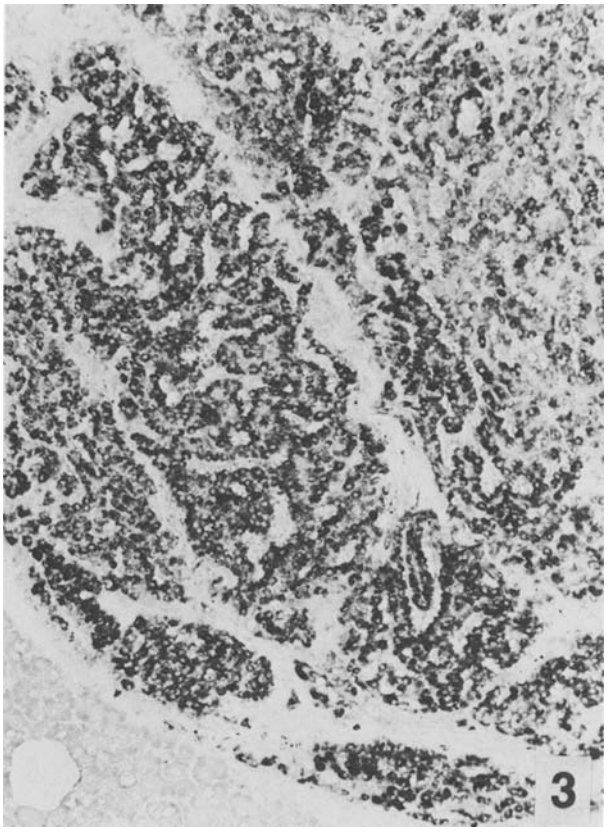


FIGURE 1.—Insulin in small normal islets and islet cell adenoma in the ACI/segHapBR rat. ABC immunocytochemistry technique; hematoxylin counterstain. × 38

FIGURE 2.—Glucagon present in small areas of islet cell adenoma and individual cells in other portions of tumor. The majority of tumor cells contained insulin. ABC immunocytochemistry technique; hematoxylin counterstain. × 38



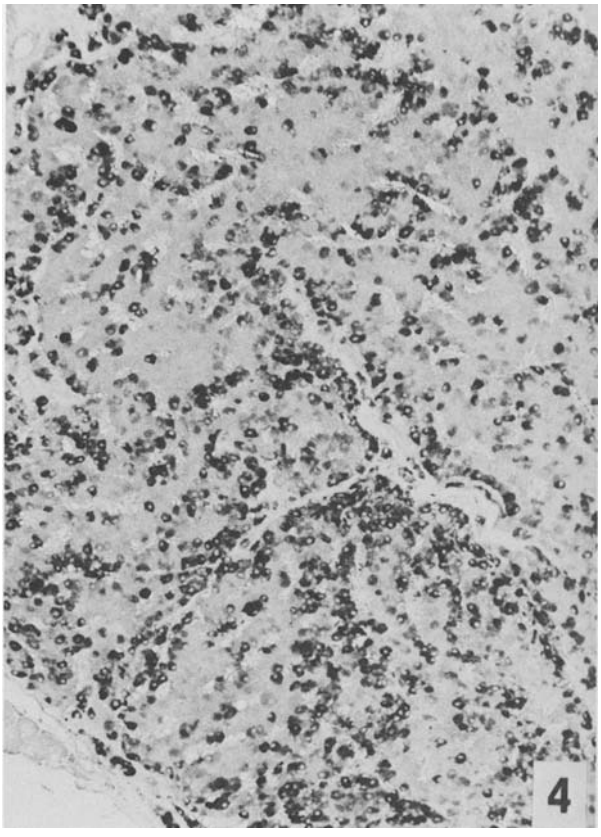


FIGURE 3.—Insulin in portion of islet cell adenoma. ABC immunocytochemistry technique; hematoxylin counterstain. × 130
FIGURE 4.—Somatostatin in tumor cells in portion of islet cell adenoma from same area as that in fig. 3. ABC immunocytochemistry technique; hematoxylin counterstain. × 130