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Spontaneous Tumors in Holtzman-Source Rats of Various Ages

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Testing of various compounds for toxicity dictates a certain familiarity with the types and incidence of spontaneous tumors which occur in the species and strain of animals used. This becomes especially important in long term tests with the albino rat because the incidence of tumors is high and tends to increase significantly with aging[6-8, 12, 21].

The incidence of tumors in large series of albino rats ranging from 2 years of age and up has been reported as 42 to 59% in the Sprague-Dawley strain[7, 29], 45% in the Osborne-Mendel strain[23], and 24 to 64% in Wistar-derived rats[5, 12, 19]. The frequency of tumors in rats of crossbred strains is apparently lower, with 1 to 6% incidence reported[6, 8].

Tumors have generally been associated with old age and infrequently observed in young and middle-aged rats. This report summarizes our experience with spontaneous tumors in a large population of Holtzman-source albino rats and emphasizes the occurrence of tumors in younger animals.

Materials and Methods

The animals used in this study were Sprague-Dawley-derived, random-bred albino rats, obtained directly from Holtzman Farms, Madison, Wisconsin. Upon receipt, they were held under uniform laboratory conditions. They were housed separately by sexes in groups of 10 per wire-mesh cage under a constant temperature of $74 \pm 1°$ F. and humidity of 55% and were given food* and water ad libitum. At the end of a 2-week holding period, when about 6–7 weeks of age and weighing

* Breeders Ration, Parke, Davis and Co.

165–185 g, they were randomly allotted to treatment and control groups for use in toxicologic studies. Animals in this study were from a number of experiments conducted over the past 4 years and ranging in duration from 1 to 18 months. During the studies, the animals were all subjected to similar environmental conditions except for the drugs administered to those in the treated groups. A total of 5086 rats (1699 females, 3387 males) was so utilized. About ¾ of these animals were treated with drug usually admixed with the ration, and the remaining ¼ was given the ration only. Our data include tumors arising in untreated rats and in treated rats whose tumors were judged by direct comparison to their respective controls to be spontaneous in origin and unrelated to administration of any drug.

The rats were killed at intervals based on condition of the animal or duration of the particular study. For ease in handling the data, observations are based on quarterly (3-month) intervals for 6 quarters. The latent period for the development of a tumor was based on the number of days the animal had been on test at the time the tumor was observed, either externally by detection based on weekly examination or at necropsy in those tumors that were internal or microscopic. The animals examined were arranged according to these intervals and to sex (Table I).

Complete necropsies on all rats were conducted after chloroform inhalation and initial perfusion with saline, followed by perfusion with 10% buffered neutral formalin. Tissues from all organs were processed through paraffin, sectioned, and stained with hematoxylin-eosin. Bone marrow was stained with Giemsa, pituitary with trichrome-PAS, and brain with thionin. Frozen sections of liver, kidney, and adrenal were stained with oil red O.

Results

Of the 5086 rats of all ages, 218 had tumors, an incidence of 4.3%. Of the females, 139 (8.2%), and of the males, 79 (2.3%) had tumors. The number of tumors increased with age, progressing from 0.14% and 0.37% incidence in males and females, respectively, during the first quarter to 25.3% and 42.2% incidence, respectively, of those examined during the last (15–18 months) quarter (Table I).

We were interested in determining what the pattern of incidence of

Table I. Number of Rats Examined for Tumors and Those with Tumors Listed According to Age and Sex

Age (Days)	Number Examined		Number with Tumors	
	Males	Females	Males	Females
<91	2127	810	3	3
92–182	454	271	2	8
183–274	234	182	8	12
275–365	325	212	35	42
366–457	164	108	10	25
458–549	83	116	21	49

tumors would have been had not so many rats in the population been removed by early autopsy from the population at risk. Consequently, to statistically estimate the tumor potentiality of the rats we adopted a 'Life Table' technic as described by MERRELL and SHULMAN[16] and as applied in a somewhat comparable situation by GELLHORN[11]. The cumulative probability of development of tumors at the end of 18 months was calculated to be 49% for males and 70% for females (Table II). These figures for incidence, although admittedly high, are not inconsistent with data derived from other populations of rats and based on the life-span of the animals.

Table II. Statistical Estimation of the Potentiality of Rats to Develop Tumors

	x*	lx	dx	cx	qx	px	Px	Qx
Males	0–91	3387	2124	2325.0	3	99.87	99.87	0.1
	92–182	1260	452	1034.0	2	99.81	99.68	0.3
	183–274	806	226	693.0	8	98.85	98.53	1.5
	275–365	572	290	427.0	35	91.80	90.45	9.6
	366–457	247	154	170.0	10	94.12	85.13	14.9
	458–549	83	62	52.0	21	59.62	50.75	49.3
Females	0–91	1699	807	1295.5	3	99.77	99.77	0.2
	92–182	889	263	757.5	8	98.94	98.71	1.3
	183–274	618	170	533.0	12	97.75	96.49	3.5
	275–365	436	170	351.0	42	88.03	84.94	15.1
	366–457	224	83	182.5	25	86.30	73.30	26.7
	458–549	116	67	82.5	49	40.61	29.77	70.2

x* = Interval of time in days
lx = No. of rats alive at beginning of period (lx+1 = lx–dx–qx)
dx = No. of rats killed during period without tumors
cx = Effective No. of rats during the period (lx–½ dx)
qx = No. of rats developing tumors during period
px = Estimated No. of rats not developing tumors during period (100(1–qx/cx)
Px = Cumulative % of rats not developing tumors (Px × succeeding px)
Qx = Cumulative % of rats developing tumors (100–Px)

Among the 218 rats with tumors, there were 29 (26 females, 3 males) with multiple tumors. There were 24 with 2 tumors each, 4 with 3 tumors each, and 1 with 4 tumors. There was, therefore, a total of 253 separate tumors (82 in males, 171 in females) originating in various sites (Table III). The majority of the tumors were benign. Single cases of tumors originating in the lung, nervous system, bone marrow, skin, and mammary gland had demonstrable metastases. Several other tumors were histologically malignant, but metastasis was not found.

Table III. Summary of Tumors According to Site of Origin and Sex

Site	Number Tumors Males	Females
Lung	1	0
Liver	2	0
Kidney	0	1
Heart	0	1
Salivary Gland	0	1
Intestine	1	0
Ovary	–	3
Uterus	–	4
Nervous System	6	0
Thyroid	7	13
Parathyroid	0	1
Pituitary	9	23
Adrenal	43	56
Bone Marrow	1	0
Skin and Appendages	9	14
Mammary Gland	1	51
Auditory Canal Gland	2	3

Brief descriptions of the types of tumors found in this survey, according to anatomic site, follow. In the literature review, particular emphasis has been given to neoplasms which were similar to those reported by other investigators in Sprague-Dawley-derived rats.

Lung

The only pulmonary tumor was a bronchogenic carcinoma in a male rat killed at 341 days of age. The tumor was composed of moderately anaplastic and pleomorphic cells with a tendency in some areas towards formation of alveoli and multinucleate giant cells; there were occasional mitotic figures. There were metastatic foci in the liver and contiguous extension to the heart, thymus, and mediastinum. Similar tumors were described by Davis et al.[7] as bronchogenic carcinomas in 2 old Sprague-Dawley rats (1 of each sex).

Liver

There were 2 hepatic tumors, both in male rats. An hepatic cell adenoma occurred in an animal killed at 228 days of age and a hemangioma occurred in an animal killed at 184 days of age. The adenoma

consisted of a minute circumscribed focus of small hyperplastic and hyperchromatic hepatocytes arranged in cords separated by sinusoidal channels. The capillary hemangioma consisted of blood-filled vascular spaces lined by a single layer of hypertrophied and hyperchromatic endothelial cells. A number of investigators[6, 23, 27] have reported hepatomas in low incidence in several strains of rats; Ross and BRAS[20] observed 3 hepatomas in Sprague-Dawley-derived rats. SNELL[27] reported 2 cases of hemangioma of the liver, occurring at 27 months of age, in inbred (NIH) rats.

Kidney

A female killed at 231 days of age had an embryonal nephroma (Wilm's tumor) composed of both epithelial and connective tissue elements. Similar tumors have been described as isolated occurrences in young rats of several strains[1, 18], including Sprague-Dawley[29].

Heart

The only cardiac tumor, in a female killed at 455 days of age, consisted of a small focus of vascular spaces lined by hyperplastic and hypertrophied endothelial cells and was classified as an endothelioma. Similar tumors have been reported in the skin[18] and lymphatic organs[27] but not in the heart.

Salivary Gland

A female killed at 117 days of age had an undifferentiated carcinoma of the submaxillary gland. It consisted of small, dark, oval to fusiform cells arranged in cords and nests which were separated by narrow bands of collagen or reticulum fibers. Mitotic figures were numerous and the tumor infiltrated adjacent tissues. A few similar carcinomas have been reported in older Wistar[12] and Sprague-Dawley[7] rats.

Intestine

A 470-day-old male rat had an adenocarcinoma of the ileum. The tumor consisted of nests, cords, or sheets of anaplastic epithelial cells

which appeared to be derived from the glands of Lieberkühn. Mitotic figures were relatively common. DUNNING and CURTIS[8] reported 2 adenocarcinomas involving the jejunum in outbred rats, and THOMPSON et al.[29] described a similar tumor of the ileum in a male Sprague-Dawley rat 22 months of age.

Ovary

A microscopic lipoma was found at necropsy in the ovary of a 306-day-old rat. This tumor was composed principally of well-differentiated lipocytes with minimal connective tissue and inflammatory cell elements. Lipoma of the ovary has not been described previously in the rat. There were grossly visible cysts in 2 other animals killed at 365 days of age. Microscopically, the cysts were identical and consisted of large cavities lined by stratified squamous epithelium.

Uterus

Four rats had well-differentiated adenomatous endometrial polyps with an average latent period of 467 days. THOMPSON et al.[29] have described similar tumors in a 2-year-old Sprague-Dawley rat, and it has been reported[27] that polyps are common in older rats of certain strains.

Nervous System

There were 3 tumors in the brain and 3 in the peripheral nervous system. They occurred in male rats and had an average latent period of 306 days. An ependymoma apparently originated from the lining of the 4th ventricle. It consisted of rounded to fusiform, deeply basophilic cells with scanty cytoplasm, had mitotic figures in some areas, was very vascular, and dilated and filled the ventricular cavity. Two rats had cerebral astrocytomas that were histologically similar. The cells were relatively uniform, and there were occasional mitotic figures. A few scattered cells had numerous round acidophilic bodies in their cytoplasm.

One rat 29 days of age had a capsular neurofibroma of the adrenal gland; it consisted of a small, well-circumscribed nodule of loosely whorled spindle-shaped cells with interlacing bundles of fibers. Another rat had a subcutaneous neurofibroma that was moderately cellular

and contained occasional mitotic figures. The cells formed interlacing, randomly arranged bundles with a suggestion of whorling or nuclear palisading in a few areas. A third rat had a retroperitoneal neurofibrosarcoma with a metastasis to the lung.

Reports of tumors of the brain in rats are rare. A glioblastoma, similar to the astrocytomas reported here, has been described in a 3-year-old Osborne-Mendel rat[23]. THOMPSON et al.[29] reported an ependymoma in a male Sprague-Dawley rat 11 months of age.

Thyroid

There were 20 follicular adenomas in the thyroid; these occurred with greater frequency in females than in males. The average latent period of the tumors was 507 days. The majority of the tumors were unilateral and consisted usually of small circumscribed foci of hypertrophic and hyperchromatic follicular cells. The foci were not encapsulated but were sharply demarcated from surrounding normal parenchyma. Four of them were in animals with other endocrine-derived tumors (1 adrenal, 1 pituitary, 2 mammary gland). Adenomas of the thyroid have been reported in other strains of rats[5, 27], as well as in the Sprague-Dawley strain[10, 29]. THOMPSON and HUNT[30] found an unusually high incidence (39%) of thyroid adenomas in rats of this strain when they examined serial sections of the gland.

Parathyroid

There was a single parathyroid adenoma in a female rat 546 days of age. It consisted of a nodule of hypertrophic, well-differentiated cells. Similar tumors have been reported, although rarely[10, 23, 27].

Pituitary

There were 32 tumors of the anterior pituitary, 23 of which were in females. The tumors had an average latent period of 398 days. Pituitary tumors accounted for 13% of all tumors in this series. Nine of the tumors were in animals with other endocrine or genital tumors (5 adrenal, 4 mammary gland, 1 each in ovary, uterus, and thyroid). One female had 2 such tumors. There were 31 chromophobe adenomas and 1 basophil adenoma. The chromophobe adenomas varied in size

from minute microscopic nodules to masses involving most of the anterior lobe. All histologic types described by WOLFE and WRIGHT[32] were represented. The basophil adenoma consisted of a small nodule of cells with distinctly PAS-positive cytoplasmic granulation.

Pituitary tumors, particularly chromophobe adenomas, are common in all strains of rats[10, 22, 25, 26, 29, 31]. In the Sprague-Dawley strain, THOMPSON and HUNT[30] observed them in animals after 360 days of age and reported an overall incidence of 16%. In contrast to our experience, males had a higher incidence than females. Basophil tumors of the anterior pituitary are not common. BIELSCHOWSKY[4] reported functional and nonfunctional tumors of similar type in conjunction with thyroid tumors in Wistar and hooded rats.

Adrenal

There were 98 rats with adrenal tumors, 91 with cortical adenomas, including 1 with a myelolipoma, and 7 with pheochromocytomas. These occurred slightly more frequently in females than males and accounted for 39% of all tumors. One male rat had 2 histologically dissimilar adrenal tumors. Otherwise, multiple tumors, when they occurred, were of the same type.

The distinction between glandular hyperplasia and adenoma is, at best, difficult and tends to be somewhat arbitrary. For our purposes, any circumscribed focus of adrenal cortical cells which were hypertrophied and hyperchromatic and which tended to compress surrounding cortical tissue or alter the normal architectural appearance of the cortex was considered to be an adenoma. Cortical adenomas developed in 91 rats (38 males, 53 females) and though the majority of these were single tumors, 10 were multiple and 3 were bilateral. Determination of latent periods was not meaningful since the adenomas varied markedly in degree of development; however, they were observed as early as 70 days. The tumors ranged from 238μ to macroscopic in diameter. Their histologic appearance varied from solid to cord-like or cystic formations, with many foci having combinations of these patterns. The tumors appeared to originate from cells of the zona fasciculata. Invariably, frozen sections stained with oil red O indicated a marked reduction of lipid in tumor cells. In one adrenal with an adenoma, an additional area in the cortex contained fat, bone, and marrow elements and was designated a myelolipoma.

Pheochromocytomas occurred in 7 animals (3 females, 4 males), with bilateral tumors in 1 animal. The average latent period of these was 492 days. Histologically, these tumors tended to be well-differentiated but varied greatly in size.

Fifteen of the animals with adrenal tumors had coincident tumors in other endocrine organs including mammary gland (8), thyroid (1), ovary (2), and pituitary (4).

"Adenoma-like nodules" and "nodular metaplasias" of the adrenal cortex have been reported to accompany aging degenerative changes[14, 15]. However, true cortical adenomas have been observed in only a few instances in Sprague-Dawley-derived rats[10, 20]. SNELL[27] has stated that cortical adenomas are particularly common in some strains of rats after 1 year of age. Pheochromocytomas are said to be the most common adrenal tumor with an incidence of up to 86% in old Wistar rats, with greater frequency in males than females, and often associated with cortical adenomas[13]. In Sprague-Dawley rats, THOMPSON and HUNT[30] reported 8% with pheochromocytomas at an average age of 22 months.

Bone Marrow

There was a myelocytoma in a male rat killed at 347 days. Histologically, there were variable foci of neoplastic cells in the liver, lung, kidney, thymus, and spleen. There appeared to be a preponderance of myeloid elements in the section of bone marrow. The neoplastic foci consisted of an admixture of large mononuclear cells and granulocytes in various stages of maturation. Mitotic figures were plentiful. Any similar lesion has not been reported in rats.

Skin and Appendages

There were 22 rats (13 females, 9 males) with tumors of the skin and/or its appendages. One female had 2 separate tumors.

Two females, at 306 and 459 days of age, had microscopic epidermal inclusion cysts, probably derived from adnexal structures, in the inguinal area. These cysts may not represent true neoplasms but such has not been previously reported in the rat.

Seven female rats had sebaceous gland adenomas in the inguinal area. They had an average latent period of 277 days but were observed

as early as 14 days in 1 animal. All were similar and consisted of focal aggregates of well-differentiated sebaceous glands, many of which had undergone cystic formation and squamous metaplasia.

In 4 other animals there were undifferentiated carcinomas of adnexal or possibly epidermal origin. These occurred in 2 rats of each sex and had an average latent period of 136 days. The tumors were all solitary and macroscopic in size. They occurred, with 1 exception, on the head or neck. They all had a similar histologic appearance but metastasis to the lung occurred in only 1 instance. The cells tended to be arranged in cords, islands, or nests, suggestive of squamous and/or basal cell carcinomas. In some sections, there was hyperplasia of basal cells of the hair follicles and of the sebaceous glands.

Carcinomas of sebaceous gland origin have been reported in single cases by DUNNING and CURTIS[8] and GILBERT and GILLMAN[12], but a benign tumor as described here has not been previously reported. Carcinomas of the skin, primarily epidermal, have been reported in other strains, as well as in Sprague-Dawley rats[20], but direct comparison to any of these is difficult because of the poor differentiation of the tumors in this group.

There were 9 rats with 10 tumors of mesenchymal origin in the subcutaneous tissue. These were predominantly in males. The tumors had an average latent period of 273 days. A single macroscopic lipoma occurred on the shoulder of a male rat. In 3 other rats (2 females, 1 male), there were 4 macroscopic fibromas located in the inguinal, axillary, or shoulder areas of the body. Two males had histologically similar fibrosarcomas in the flank and sublingual areas, respectively. The tumors were highly cellular and contained numerous mitotic figures. The tumors from the remaining 3 rats in this group were unclassified mesenchymal tumors in male rats, were macroscopic, and were located on the shoulder or in the flank. Histologically, cellular degeneration and/or necrosis made their classification difficult. Mitotic figures were infrequent and the general histologic appearance did not suggest a high degree of malignancy. These neoplasms may represent degenerating fibrosarcomas.

Most of the tumors of the subcutaneous tissue as reported here have been described in various populations of rats. Lipomas[20], fibromas[20], and fibrosarcomas[20, 29] have been reported in Sprague-Dawley rats. Mesenchymal tumors have been observed infrequently with a few reported by SAXTON et al.[23] in Osborne-Mendel rats and by CRAIN[5] in Wistar-derived rats.

Mammary Gland

The 52 tumors of the mammary gland occurred in 46 rats and all but 1 were in females. The average latent period of the tumors was 365 days (range 138–548 days). The tumors were of variable size and were located primarily in the inguinal and axillary areas, although several were located on the neck and dorsum. Twelve were found in animals with other endocrine tumors (4 pituitary, 8 adrenal, 2 thyroid).

Neoplasms in this series were classified according to the predominant cell type and included 28 adenofibromas, 21 fibroadenomas, 2 adenocarcinomas, and a fibroma. One of the adenocarcinomas had metastasized to the lung.

According to the excellent review of NOBLE and CUTTS[17], mammary gland tumors are common in rats and account for 6 to 77% of all spontaneous tumors. In this series, they accounted for almost 21% of all tumors. They have been noted to occur as early as 20 weeks of age, which parallels our findings, but the majority develop after 1 year of age. In this series, the overall incidence of female rats with mammary tumors was 2.7%, a relatively low frequency. However, when incidence of tumor was corrected for minimal age of their development (at 138 days), the incidence was about 6%. This frequency is low compared to the incidence of 12 to 62% reported for Sprague-Dawley females at an average age of 22 months[2, 3, 7, 9, 29]. Assuming that the incidence of mammary tumor in Sprague-Dawley female rats and its sublines be a characteristic which has remained stable through many generations[9], we must conclude that our data in young and middle-aged rats do not accurately reflect the incidence of mammary tumors in rats of this strain. Indeed, incidence of mammary gland tumors over the life-span and median age of onset are said to be the most reliable criteria of the ultimate incidence of mammary tumors in a population of rats[9].

Auditory Canal Gland

There were 5 tumors in the auditory canal gland. Three occurred in females and 2 in males, at an average age of 408 days. The tumors ranged from microscopic to macroscopic.

These have been described previously in some detail[24]. The tumors were designated adenomas and arose as nodules in deeper portions of the lobules of cystic glands of the auditory canal. Similar

tumors have been reported only rarely in Sprague-Dawley rats[24, 28] and in rats of other strains[27].

Discussion

The pattern of development of tumors in our young and middle-aged rats compares favorably in certain aspects with reports of spontaneous development of tumors in old rats of several other strains. Overall incidence, 4.3%, was low, but was expected, based on the view of Ross and Bras[20] that increased rates of incidence for development of tumors are exponential and are a function of the logarithm of age up to about 2.5 years. When corrected for extraneous deaths and other factors, however, the potential for development of tumors was 49% for males and 70% for females. These frequencies are comparable to rates of incidence reported previously for this strain, 13 to 37% for males[7, 20, 29] and 44 to 66% for females[7, 29].

Many of the types of tumors which we encountered have been described frequently in other reports. Exceptions included bronchogenic carcinoma, hepatic cell adenoma and hemangioma of the liver, endothelioma of the heart, carcinoma of the salivary gland, adenocarcinoma of the intestine, lipoma of the ovary, tumors of the brain, basophil adenoma of the pituitary, parathyroid adenoma, myelocytoma, sebaceous gland adenomas, carcinomas and mesenchymal tumors of the skin, adenomas of the auditory canal gland, myelolipoma of the adrenal, and adrenal cortical adenomas.

An important feature described in this study was the spontaneous occurrence of many tumors in young rats. Tumors have not been previously reported in rats prior to puberty; the earliest tumor thus far described was a renal carcinoma in a 5-month-old female Wistar-derived rat[6]. In our study, a few tumors were observed in rats less than 3 months of age. These included tumors of the skin appendages, adrenal, and nervous system. In addition, tumors of the salivary gland, brain, adrenal, skin and/or skin appendages, and mammary gland were observed in rats less than 6 months old. This observation supports the contention of Gilbert and Gillman[12] that neither a decline in sexual activity nor age per se determine the susceptibility to neoplasia.

The majority (83%) of tumors in this series did occur after 9 months of age and increased in frequency up to 18 months. This association of the development of tumors with aging has been attested

to by a number of investigators[6–8, 12, 21]. Indeed, in Wistar rats, the mean age of rats bearing tumors in a large population was 18 months for males and 22 months for females, with the greatest incidence at 15 and 21 months, respectively[6]. DAVIS et al.[7] reported that 87% of the tumors in their Sprague-Dawley rats occurred after 18 months of age.

Earlier reports of spontaneous tumors in rats have emphasized development of tumors over the lifetime of the animal. Our results suggest that difficulty might arise in the interpretation of a possible carcinogenic effect of a compound during relatively short term experiments in rats unless more comprehensive data on spontaneous development of tumors in younger animals of a given strain be obtained.

Summary

Spontaneous development of tumors in 5086 Holtzman-source rats ranging in age from 1 to 18 months is reviewed. Overall incidence of tumors was 4.3% (8.2% for females, 2.3% for males). Statistical analysis of the data demonstrated that the potentiality for tumors was comparable to previously reported lifetime studies. Characteristics of development of tumors are given, and the more uncommon tumors are described briefly. The occurrence of tumors in young rats in this survey indicates the need for accumulating data on tumors in other than "old" rats as an aid in interpreting data obtained from toxicological studies.

Zusammenfassung

Es wird zusammenfassend über die Entwicklung von spontanen Tumoren bei 5086 Holtzman Ratten im Alter von 1–18 Monaten berichtet. Das Gesamt-Tumor-Vorkommen betrug 4,3% (8,2% bei Weibchen, 2,3% bei Männchen). Die Analyse mit Hilfe der «Life Table» zeigte, dass die Potenz der Tumorbildung mit früher geschilderten Lebenszeit Untersuchungen vergleichbar ist. Charakteristische Eigenschaften der Tumorentwicklung werden geschildert und besonders ungewöhnliche Tumoren kurz beschrieben. Das Auftreten von Tumoren bei jungen Ratten aus dieser Untersuchungsreihe zeigt, wie notwendig es als Hilfe für die Interpretation toxikologischer Untersuchungen ist, nicht nur bei «alten» Ratten Tumoren zu registrieren.

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