The Incidence of Spontaneous Hepatomas in C3H, C3H (Low Milk Factor), and CBA Mice and the Effect of Estrogen and Androgen on the Occurrence of These Tumors in C3H Mice*

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Andervont (2) has recently reviewed the literature on the occurrence of spontaneous hepatomas in C3H and CBA mice. It is now well established (1, 3, 6, 10, 13, 14) that mice of these strains may develop spontaneous hepatomas and that these tumors most commonly arise in older mice, i.e., those more than 1 year old. Furthermore, many previous studies (2, 4–7, 9, 10) on C3H and CBA mice have indicated that spontaneous hepatomas occur more often in males than in females; conversely, Andervont (2) has reported that there was a lower incidence of these tumors in castrated C3H males than in intact litter-mates.

The relationship between hormonal stimulation and the development of spontaneous hepatomas in mice has also received attention. Shimkin and Wyman (12) decreased the incidence of spontaneous hepatomas in C3H males by giving estrogen. However, Burns and Schenken (4) and Schenken and Burns (11) found that estrogens increased the incidence of these tumors in C3H males and decreased the incidence in C3H females; and, of 48 C3H males and females treated with testosterone propionate, only one female developed a hepatoma. Again, although Miller and Pybus (8) decreased the incidence of hepatomas in castrate CBA males by giving estrogen, they also reported (10) that there was no diminution in the incidence of hepatomas in intact CBA males injected with estrone. Thus, as Andervont (2) has pointed out, "It is clear, as indicated by all these investigators, that further efforts along these lines are essential."

Nearly 15 years ago an equal sex distribution of this tumor was reported (15) from this laboratory, and Andervont (2) recently stated that this "represents the only claim, thus far, for an equal sex distribution of this tumor." It seemed of interest, therefore, to compare the incidences of spontaneous hepatomas according to sex in C3H, C3H (low milk factor), and CBA mice examined since that time, and also to present data concerning the possible effect of estrogen and androgen on the incidence of these tumors in C3H mice.

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MATERIALS AND METHODS

The mice used in these experiments were reared and maintained under suitable conditions in this laboratory. Ample food and water were always available. Fox chow was used until 1948, and laboratory chow (Purina) thereafter; it is doubtful whether this relatively recent change in diet has significantly affected the result of these experiments. At autopsy, the presence of hepatomas in mice that had died or had been killed was usually determined by gross examination, as the appearance of a hepatoma is usually characteristic (2, 14). However, in doubtful cases and in a few characteristic cases, the suspect tissue was examined histologically.

The incidence of spontaneous hepatomas was recorded for three groups of mice:

Group 1: stock breeders.—Male and female C3H, C3H (low milk factor) and CBA mice that were at least 300 days old when killed or found dead constituted this group. The number of mice used of each strain and the sex are indicated in Table 1.

Group 2: estrogen-injected.—Male and female mice of the C3H strain that had been injected weekly or fortnightly with varying amounts of estrogen (usually estradiol benzoate dissolved in sesame oil) for varying periods and that were at least 300 days old when killed or found dead made up this group. The number of mice used of each sex, the amount of estrogen given, and the duration of estrogen administration are indicated in Table 2.

Group 3: androgen- and estrogen-injected.—Male and female mice of the C3H strain that had been injected weekly with varying amounts of testosterone propionate and estrogen (usually estradiol benzoate dissolved in sesame oil) for varying periods and that were at least 200 days old when killed or found dead were included in this group. This group had been set up for another purpose, but from the standpoint of the effect of these hormones on the occurrence of hepatoma, with one exception (see footnote to Table 3), enough testosterone was

TABLE 1

Incidence of Hepatomas in Untreated C3H (High and Low Milk Factor and CBA Mice More than 300 Days Old

Strain	No. and sex of mice	Incidence of hepatomas in different age groups: Hepatomas/no. of mice (days) 300–400	401–500	501–600	601–700	701–800	>800	No. of hepatomas/no. of mice in different age groups (Per cent incidence in parentheses) >300	>400	>600
CBA BrC	88♂	0/9	1/21	4/20	6/23	1/9	3/6	15/88(17)	15/79(19)	10/38(26)
"	92♀	1/29	0/20	0/15	1/10	3/15	0/3	5/92(5)	4/63(6)	4/28(14)
C3H BrC High Milk Factor	156♂	1/33	1/41	7/52	5/27	1/2	0/1	15/156(10)	14/123(11)	6/30(20)
"	192♀	0/113	1/51	0/15	0/11	0/2		1/192(0.5)	1/79(1)	
C3H BrC Low Milk Factor	80♂	1/19	1/18	3/33	0/9	0/1		5/80(6)	4/51(8)	
	216♀	0/56	1/71	1/61	0/27	0/1		2/216(1)	2/160(1)	
Total	824–324♂ 500♀	3/259	5/222	15/196	12/107	5/30	3/10			

TABLE 2

Incidence of Spontaneous Hepatomas in C3H Mice That Had Received Varying Amounts of Estrogen and That Survived 300 Days or More

	No. and sex of mice	Estrogen (μg/week)	Mean duration of treatment in weeks (range in parentheses)	No. of hepatomas in mice >300 days old	Incidence of hepatomas in different age groups: Hepatomas/no. of mice (days) 300–400	401–500	501–600	>600
	12♂	1	74 (52–93)	3	0/1	1/1	1/4	1/6
	12♂	3.3	76 (37–114)	1	0/3	0/3	1/1	0/5
	10♂	8.3	48 (39–64)		0/7	0/2	0/1	
	16♂	10	58 (37–76)		0/3	0/7	0/6	
	3♂	16.6	41 (32–48)		0/3			
	27♂	25	53 (38–74)		0/13	0/12	0/2	
	4♂	33.3	42 (32–58)		0/3		0/1	
	3♀	3.3	69 (61–85)			0/2		0/1
	1♀	8.3	34		0/1			
	4♀	16.6	36 (32–48)		0/4			
	3♀	33.3	42 (39–43)		0/3			
Total	84♂		56 (32–114)	4♂	0/33	1/25	2/15	1/11
	11♀		45 (32–85)	0♀	0/8	0/2		0/1

TABLE 3

Incidence of Hepatomas in C3H Mice That Had Received Varying Amounts of Both Estrogen and Androgen and That Had Survived 200 Days or More

No. and sex of mice	Amount of estrogen weekly (μg.)	Amount of testosterone weekly (mg.)	No. of hepatomas in mice >200 days old	Incidence of hepatomas in different age groups: Hepatomas/no. of mice (days) 200–400	401–600	601–800
7♂	16.6	0.625*	1	0/1	1/6	
4♀	"	"		0/1	0/3	
15♂	16.6	1.0	2	0/10	2/3	0/2
5♀	"	"	2	0/2	0/1	2/2
1♂	33.3	"		0/1		
11♀	"	"		0/6	0/5	
18♂	16.6	1.0 for 12 wks.	1	0/4	1/13	0/1
35♀	"	1.25		0/12	0/22	0/1
18♂	"	1.25 for 10 wks.	2	0/4	2/14	
2♂	33.3	1.25		0/2		
1♀	"	"		0/1		
5♂	16.6	2.5	1	0/3	1/2	
16♀	"	"		0/11	0/5	
4♂	33.3	2.0	1	0/1	1/3	
1♀	"	"		0/1		
Total 70♂			8♂	0/26	8/41	0/3
73♀			2♀	0/34	0/36	2/3

* Testosterone not physiologically dominant at this dose level.

given to nullify the effect of the relatively small dose of estrogen that was given simultaneously. Usually the injections started when the ages of the mice ranged from 35–66 days, though in one instance they were started as early as the 24th day and in another as late as the 114th day. The number of mice used at the amounts of androgen and estrogen given are shown in Table 3.

RESULTS

GROUP 1

Stock breeders, C3H strain.—Hepatomas were found in fifteen of 156 (10 per cent) male mice that survived at least 300 days (Table 1). Only one hepatoma, however, was observed in a group of 192 females that had survived 300 days or longer. A breakdown of these figures (Table 1) showed that the percentage of hepatomas (No. of mice with tumors/No. of mice, ×100) tended to rise with increasing age. For all male C3H mice surviving at least 300 days, the incidence was 10 per cent. In the group surviving 400 days or longer, the incidence was 11 per cent; while six of 30 males (20 per cent) in the group surviving >600 days had hepatomas. The solitary tumor observed in the females gave an incidence of 0.5 per cent in the group of 192 that were at least 300 days old and a 1 per cent incidence for the 79 that were >400 days; no hepatomas were observed in the >600-day category. The marked sex difference in incidence of these hepatomas is shown graphically in Chart 1.

C3H (Low milk factor) strain.—Here again, a sex difference was observed (Table 1). Five of 80 (6 per cent) males that survived at least 300 days had hepatomas, but only two of 216 (1 per cent) females in the same age group. Although no hepatomas occurred in males or females in the >600-day category, the tendency toward a higher tumor incidence with increasing age was again observed, but only in males; four of 51 (8 per cent) males in the >400-day category had hepatomas. Chart 1 indicates graphically the sex difference in occurrence of hepatomas and the tendency of increasing age to favor a high tumor incidence in the males of this group.

CBA strain.—These results (Table 1) were similar to those found in Groups 1 and 2—a predominantly male sex incidence of spontaneous hepatomas and a tendency for more tumors to arise in the older animals. Hepatomas were observed in fifteen of 88 (17 per cent) of the male mice that survived at least 300 days but in only five of 92 (5 per cent) comparable females. Fifteen of 79 (19 per cent) males in the >400-day category had tumors, compared to four of 63 (6 per cent) females in the same age group; and in the >600-day category, hepatomas were found in ten of 38 (26 per cent) males and in four of 28 (14 per cent) females. These results are indicated graphically in Chart 1.

GROUP 2

Estrogen-injected C3H mice.—The results are summarized in Table 2. Four hepatomas were observed in 56 male mice, 300 or more days old, that had received amounts of estrogen varying from 1 μg. to 33.3 μg. per week. These tumors, however, only occurred at dosage levels of 1 μg. and 3.3 μg. per week; no hepatomas were found in ten male mice that had received 8.3 μg. per week, in sixteen at 10 μg. per week, in three at 16.6 μg. per week, in 27 at 25 μg. per week, or in four at 33.3 μg. per week. No hepatomas were seen in eleven females that had received amounts of estrogen ranging from 3.3 μg. to 33.3 μg. per week for varying periods.

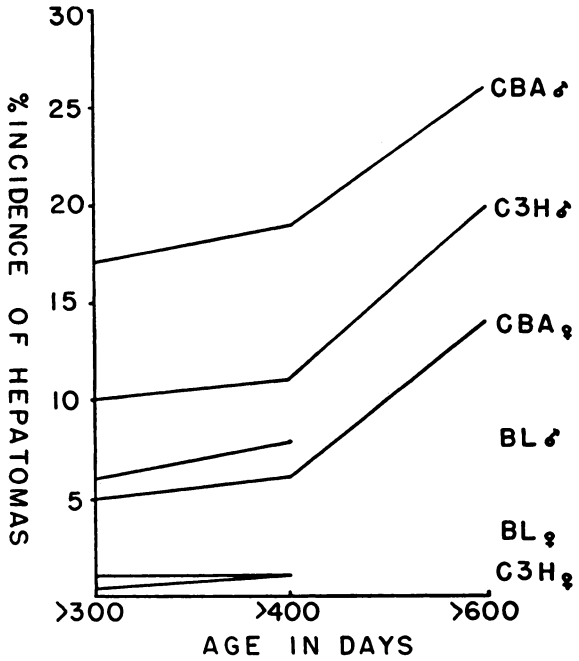

CHART 1.—Per cent incidence of hepatomas in untreated C3H, C3H (low milk factor) (designated BL in Chart), and CBA mice after 300, 400, and 600 days.

GROUP 3

Androgen- and estrogen-injected C3H mice.—The results are summarized in Table 3. As mentioned above, although both androgen and estrogen were injected, the amount of testosterone injected was physiologically dominant, except, perhaps, at the 0.625 mg/week level. Eight of 70 (11 per cent) males receiving varying amounts (Table 3) of these hormones developed hepatomas. Two of 73 (3 per cent) similarly treated females also had hepatomas; both animals belonged to a group of five that had been injected weekly with 16.6 μg. of estrogen and 1 mg. of testosterone.

DISCUSSION

Hepatomas were more common in males than in females for all the strains (C3H, C3H [low milk factor], and CBA) observed (Table 1, Chart 1). This confirms the findings of several workers (2, 4–7, 9, 10) who used C3H or CBA mice, and of Andervont (2) who also studied C3H (low milk factor) mice. Why hepatomas previously (15) developed "at approximately equal rates" in our male and female CBA mice and now show a marked preference for the male is not known. The original paper was based on the first hepatomas, 42 in all, observed in CBA mice in this laboratory; factors such as the age of the mice under observation and the total number of mice of each sex used may have influenced the results obtained.

In the present series of experiments no hepatomas were seen in mice under 300 days old, the youngest mouse with a histologically proved hepatoma being a female CBA, 302 days old. Except in Group 3 (androgen- and estrogen-injected) where the base-line was lowered to 200 days, only mice 300 or more days old were used in this study, as it would be misleading, for assessing the incidence of hepatomas, to include mice too young to develop such tumors. Generally, more hepatomas occurred in older mice, especially those in the >500-day category, and the percentage of incidence of these tumors tended to increase with age (Table 1 and Chart 1); only the females of strains C3H and C3H (low milk factor) failed to show this trend.

The presence or absence of the milk factor did not appear to affect significantly the occurrence of hepatomas in C3H mice. Fifteen of 156 (10 per cent) male C3H mice developed hepatomas, compared to five of 80 (6 per cent) male C3H (low milk factor) mice; and one of 192 (0.5 per cent) female C3H mice had tumors, compared to two of 216 (1 per cent) female C3H (low milk factor) mice. Failure of sufficient numbers of C3H females to reach the "hepatoma age" because of deaths due to mammary carcinoma is thus unlikely to be responsible for the low incidence of spontaneous hepatomas in these mice. Andervont has also reported (2) that the presence of the milk factor is not essential for the development of hepatomas in C3H mice. However, we observed fewer hepatomas in our C3H and C3H (low milk factor) mice than he did in his colony. He reported (2) 42 hepatomas in a series of 362 female C3H (without milk factor) mice and nine hepatomas in another series of 179 similar mice; for female C3H (with milk factor) mice he reported (1) an incidence of 11.7 per cent. In one series of male C3H (without milk factor) breeders he found (2) hepatomas in fourteen of seventeen. Again, in another paper (3), he recorded that, of 141 female C3H (with milk factor) mice over 1 year of age, 9.93 per cent developed hepatomas; and, of 320 similar males, 26.87 per cent had such tumors.

Studies on the effect of estrogen and androgen on the development of hepatomas (Tables 2 and 3) indicate that these hormones may modify the incidence of these tumors in male and female C3H mice. The results summarized in Table 2 suggest that estrogen in sufficient dosage can prevent or at least modify the occurrence of hepatomas in male C3H mice, as hepatomas were only found in males that had received very small doses of estrogen (1 μg. or 3.3 μg. per week) and did not occur in ten mice that had received 8.3 μg. per week, in sixteen at 10 μg. per week, in three at 16 μg. per week, in 27 at 25 μg. per week, or in four at 33.3 μg. per week. However, 10 μg. per week of estrogen, although apparently able to prevent the occurrence of hepatomas in our C3H males, may not be enough for male mice of other strains; for example, two hepatomas were observed in a group of seven CBA males that had received 10 μg. of estrogen per week for a mean period of 58 weeks. Both these mice were in the 500–600-day category when examined.

The results of the administration of testosterone to female C3H mice were less convincing (Table 3). Only two hepatomas were found in a group of 73 females, both animals belonging to a group of five that had been given weekly injections of 16.6 μg. estrogen and 1 mg. of testosterone; as mentioned above, the testosterone was physiologically dominant at this dose level. As the incidence of hepatomas in these 73 C3H females was 2.7 per cent compared to 0.5 in the group of 192 stock C3H females (Table 1), we believe that the results are at least suggestive that testosterone may increase the incidence of hepatomas in female C3H mice; certainly, further work with larger numbers of animals would seem justifiable.

SUMMARY

Hepatomas were observed in male and female mice >300 days old of strains C3H, C3H (low milk factor, and CBA, and occurred more often in males than in females. The percentage incidence of hepatomas tended to rise with the age of the mice, except in female C3H and C3H (low milk factor) mice.

Estrogen appeared to prevent the occurrence of hepatomas in male C3H mice if 8.3 μg. or more of estrogen was injected weekly for long periods. Conversely, injection of female C3H mice with testosterone seemed to increase their susceptibility to development of spontaneous hepatomas.

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