

The Development of Liver Tumors in Goldthioglucose-treated CBA Mice*

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SUMMARY

The incidence of hepatomas was markedly increased in goldthioglucose-obese CBA males as compared with nonobese controls. Goldthioglucose-induced obesity did not have as striking an effect on the incidence of liver tumors in females when compared with the males, even though the females weighed more than the treated males. Hepatomas appearing in obese intact females and castrated obese and control animals were associated with androgen-secreting adrenal adenomas. Obesity as induced by goldthioglucose is not a sufficient stimulus per se to enhance the development of hepatomas in CBA mice, but must act in association with an androgenic stimulus.

Numerous investigators (1, 2, 5, 9, 17) have described spontaneous tumors of the liver in CBA and C3H mice, and it has been suggested that the appearance of hepatomas in these two particular strains is related to their common ancestry (16). Histologically, these tumors rather closely resemble the structure of the normal liver and can be transplanted in only a comparatively low percentage of cases (3, 4, 18). Sex hormones have been considered as important factors in the genesis of these tumors, since males have a higher incidence than females (6, 7, 11, 15). However, the administration of estrogens and androgens has given inconclusive evidence regarding a specific role of sex hormones in the neoplastic transformation.

The responsiveness of the liver to altered dietary states has suggested the importance of nutritional factors in the development of hepatomas in laboratory animals and man. Tannenbaum and Silverstone (19) have demonstrated that a restriction of caloric intake decreased the incidence of hepatomas as well as several other types of tumors in mice. Such studies suggest that, conversely, "over-nutrition" might enhance tumorigenesis. Waxler suggested that the "obesity" induced with goldthioglucose was the basis for the increased incidence of hepatomas in obese C3H males (20) and for acceleration in the time of appearance of mam-

mary tumors in the females (21). The appearance of the mammary tumors at an early age in this strain precluded a study of hepatomas in the females. This information would be of interest in view of the sexual differences associated with the spontaneous appearance of hepatomas in the C3H strain.

Liebelt and Perry (13) have found that 100 per cent of CBA mice become obese following a single injection of goldthioglucose. The females of the subline used by these authors have a very low incidence of spontaneous mammary tumors, and it was the purpose of this experiment to study the effects of goldthioglucose-induced "obesity" on the occurrence of hepatomas in *both* male and female CBA mice.

MATERIALS AND METHODS

Mice of the CBA strain used in these experiments had been inbred in this laboratory by brother-sister matings for over twenty generations. This subline of the CBA strain has a low incidence of spontaneous mammary tumors (less than 1.0 per cent) and is believed to be free of the mammary tumor agent. Four to six animals were housed in wooden boxes (6" × 6" × 12") and fed Purina Laboratory Chow ad libitum. Males and females were separated at weanling age to prevent breeding and given injections of 0.4 mg/gm body weight of goldthioglucose (Solganol B)¹ in saline

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solution intraperitoneally at 90–100 days of age. Animals were divided into the following groups: (I) intact males and females, untreated; (II) intact males and females, goldthioglucose-treated; (III) castrated males and females, untreated; and (IV) castrated males and females, goldthioglucose-treated.

Castration was performed approximately 1–2 weeks prior to the injection of goldthioglucose. All animals were autopsied at 13 months of age. In doubtful cases histological confirmation of the gross diagnosis of hepatoma was made; but, in general, all hepatomas were greater than 5 mm. in diameter. Histological sections were made of the

hepatomas had long, thin uteri as well as adrenal cortical adenomas. Histologically, these adenomas were of the “A” and “B” cell type as described by Woolley and Little (22). On the basis of the histological appearance of the kidneys and submaxillary glands (12), along with the gross appearance of the reproductive tract, it appeared that the adrenal tumors were secreting androgenic steroids. The vaginas did not show epithelial cornification. No gross adrenal cortical adenomas were found in any other intact male or female (obese or nonobese) animals killed at 13 months. All males had stimulated seminal vesicles, and the uteri of all females with the exception of the two just noted were

TABLE 1
INCIDENCE OF HEPATOMAS IN GOLDTHIOGLUCOSE-TREATED CBA MICE AT 13 MONTHS OF AGE

Group	Sex	Av. wt.	No. animals	No. with hepatomas	Per cent hepatoma incidence	Animals with hepatomas and adrenal tumors	Type of sex steroid secretion of adrenal tumor
Controls	Males	35	36	7	19	0	
	Females	33	40	0	0	0	
Treated	Males	48	34	21	62	0	Androgen
	Females	53	23	2	9	2	
Castrated untreated	Males	35	16	1	7	1	Estrogen (?) Androgen plus estrogen
	Females	36	15	2	13	2	
Castrated treated	Males	49	25	5	20	5	2—Estrogen (?) 3—Androgen Androgen plus estrogen
	Females	51	22	3	13	3	

adrenal glands, kidneys, and submaxillary glands of all castrated animals with liver tumors.

RESULTS

The incidence of hepatomas in intact goldthioglucose-treated males was 62 per cent as compared with 19 per cent in intact control males (Table 1). Fewer tumors (20 per cent) appeared in obese males following castration, as was true for castrated nonobese males (7 per cent). Hepatomas did appear in some females, but each group was found to have fewer hepatomas than the corresponding group of males with the exception of the castrated nonobese group. Intact obese females had a 9 per cent incidence; no tumors (0 per cent) appeared in the intact female controls. Both the castrated-obese and castrated controls had the same incidence of hepatomas (13 per cent). The incidence of hepatomas in CBA mice bred in this laboratory and permitted to live out their lifespan (18–24 months) is approximately 18 and 6 per cent in males and females, respectively.

The two obese intact females that developed

stimulated. Frantz and Kirschbaum (8) reported that adrenal cortical adenomas in intact CBA mice are extremely rare at 13 months of age.

The CBA strain of mice develop functional adrenal cortical adenomas following castration (8). The eleven castrated males and females that developed liver tumors also had adenomas of the adrenal cortex. Gross observations and histological studies indicated that these adenomas were secreting sex steroids. Two of the castrated-obese males and one castrated control male had grossly atrophic seminal vesicles, and the histology of the dimorphic glands was of the female type. Three castrated-obese males had both gross and histological evidence of androgen activity. The five castrated (obese and control) females showed definite evidence of secretion of both types of sex hormone. In these animals the salivary glands were completely male-like, whereas the epithelium of the vaginas was cornified (8).

Adrenal cortical adenomas were grossly visible in the majority of the castrated animals that did not develop hepatomas. No histological studies

were carried out on these animals which would have permitted a more accurate determination of adenoma incidence as well as the type of sex steroid being secreted. Grossly, the seminal vesicles of the males were consistently atrophic, whereas the uteri of all the females were stimulated.

DISCUSSION

Hepatomas appear "spontaneously" in mice of the CBA strain, with males having a higher incidence than females. Thus, sexual as well as genetic factors must be considered when studying the genesis of these neoplasms.

The increased incidence of hepatomas in goldthiogluco-se-treated CBA males is in agreement with the results obtained in goldthiogluco-se-obese C3H mice (20). On the other hand, "obesity" did not have as striking an effect on the incidence of liver tumors in the females as observed in the males, especially since the body weight gains were greater in the females. Likewise, there was a markedly decreased incidence of hepatomas in the castrated-obese males.

The two treated females in which hepatomas did appear had androgen-secreting adrenal adenomas at autopsy. The hepatomas that appeared in the eleven castrated animals also were associated with functional adrenal cortical adenomas. Gross and histological evidence indicated that androgenic or a combination of androgenic and estrogenic steroids were being secreted in eight of these animals. The other three animals (males) had atrophic seminal vesicles and a female type of histology in the kidneys and salivary glands. However, a castrated male mouse will show a similar type of histological picture within 4 weeks following castration (8). Thus, with the possible exception of three instances, androgenic activity was associated with the appearance of hepatomas in castrated animals at autopsy. Frantz and Kirschbaum (8) reported that adenomas of the adrenal cortex in CBA mice did not become functional until 6-7 months following castration. This may raise some question as to the possible role played by these adrenal steroids in the incidence of hepatomas seen in this experiment. Further investigations of the relationship between adrenal cortical adenoma activity and liver tumorigenesis is warranted.

The data indicate that the enhancement of liver tumorigenesis in goldthiogluco-se-obese animals is associated with an androgenic environment, and "obesity" per se is not a sufficient stimulus to enhance or initiate a neoplastic transformation in the mouse's liver.

The mechanism by which goldthiogluco-se in-

creases the incidence of hepatomas in intact males remains to be investigated. Goldthiogluco-se may have a direct effect on the liver parenchyma, since "fatty livers" are often seen in goldthiogluco-se-obese CBA male and female mice. It is known that single injections of goldthiogluco-se into mice produce consistently localized lesions in the hypothalamus and other regions of the brain (13, 14). The hypothalamic lesions are believed to produce a hyperphagia which is considered the basis for the development of the massive, persistent obesity observed in treated animals. CBA mice injected with the dose used in this experiment cease to breed prior to the manifestation of the obesity, although the females do not show any gross aberrations in the vaginal cycle until 2-3 months following injection (Liebelt, unpublished). It is well documented that hypothalamic-pituitary interrelationships are important in the regulation of endocrine function (10), and the results of this experiment may reflect some altered hormonal mechanism that is acting independently of or in conjunction with the increased caloric intake.

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