Obesity and Cancer Susceptibility in Mice

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IN 1949 Brecher and Waxler were first to report the experimental production of obesity in mice induced by a single injection of aurothioglucose. Mice so treated developed varying degrees of true obesity. Weights of 60 to 80 gm. in males and 50 to 60 gm. in females were observed[1] (Figs. 1A, B and 2). Autopsies of these animals and chemical analyses of total body lipids, proteins, water and ash indicated that the gain in weight were primarily due to an increase in adipose tissue.[1] Some increase in the weights of the organs of obese animals was also apparent in the wet, dry and defatted states.[2]

During the period of development and maintenance of obesity the *ad libitum* consumption of food of "gold-treated" mice exceeded that of the control animals (Fig. 3). However, this obesity was readily regulated since such animals given a diet identical in caloric value to that of the control mice maintained the same weight levels as those of the control mice.[3]

Obese animals were able to mobilize their excess fat and withstand starvation for a period of from thirteen to seventeen days, while the control mice were able to endure starvation for about five to six days only. Subsequent feeding again produced obesity at the prestarvation level in animals starved down to their original weight. This indicated that the hypothalamic "lesion factor" induced by gold thioglucose was a permament one.[3]

The relationship of tumorigenesis to diet has been investigated by many workers during the past several years. The results of overfeeding and underfeeding experimental animals have generally indicated that diet has some effect on the development of both spontaneous and experimentally induced neoplasia.[4-10] Experimental evidence has been produced to show that a diet with a high fat content tends to promote or enhance the formation of many types of tumors, whereas a restricted food intake induces a delay in such formations. However, in most of these experiments comparisons were made between animals fed restricted or modified diet and those with unrestricted food intake. From such experiments we believed that no definite conclusions could be drawn as to the effect of obesity on tumor formation because the animals could not be justifiably termed truly obese. In our experiments true obesity was readily induced in the animals by an injection of gold thioglucose, and it was subsequently eliminated or reproduced by regulating the animal's food intake. These specific characteristics gave us a tool by which we could study the effects of obesity on the occurrence of spontaneously developing tumors in various strains of mice.

We produced evidence that spontaneous mammary tumors appeared earlier and in greater numbers in virgin mice of the C_3H strain made obese by an injection of gold thioglucose than did tumors in control mice of normal weights. The average age of the obese mice at tumor onset was 242 days compared to 303 days in the control animals (Fig. 4). Similarly, early occurrence of tumors was apparent in mice made previously pregnant as well as in the virgin mice. Gold-treated animals which did not become obese had the same incidence and rate of tumor appearance as did untreated mice.[11] Subsequently, we demonstrated that mice made obese and later reduced and maintained at the weight level of the control animals by paired feeding showed a slower rate of tumor

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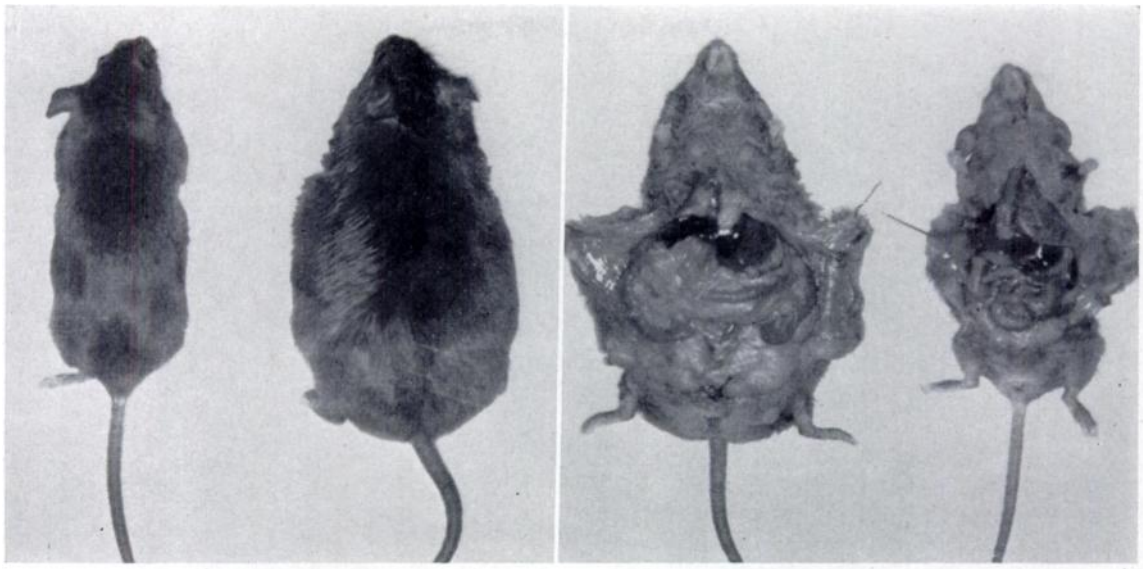

A B

FIG. 1. A, control C_3H mouse (37 gm.) and obese C_3H mouse (68 gm.). B, the same animals as in A, opened to show viscera.

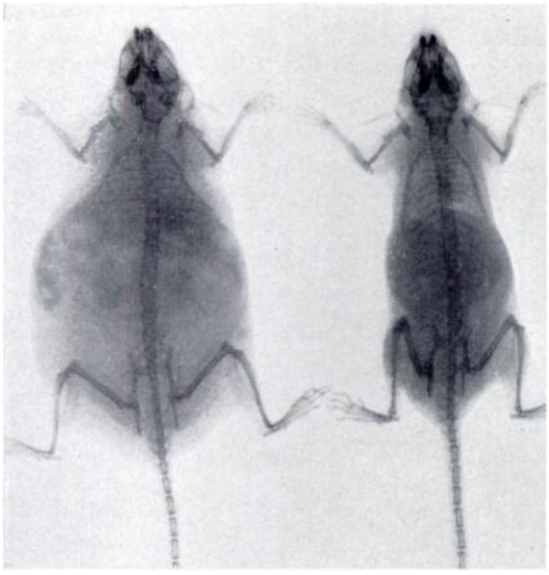

FIG. 2. Roentgenogram of obese and control mice.

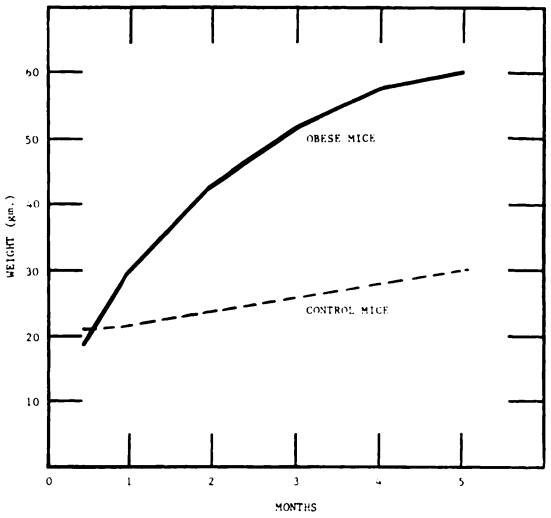

FIG. 3. Average weight gains of "gold-treated" and control mice over a five-month period.

incidence than did the control mice.[12] In this particular experiment the average number of days before tumor appearance in 50 per cent of the obese mice was 300 days, in the control mice 367 days and in the previously obese mice more than 400 days. Tumor incidence in previously obese animals was significantly less than that in their pair-fed partners, and much less than that in the obese group. In fact, after nearly all the control and obese mice were dead, a high percentage of reduced animals were still alive and tumor free.

It is well established that the appearance of mammary tumors in C_3H mice is associated with the presence of a mammary tumor agent or milk factor.[13] In control groups of C_3H mice lacking this agent, which we denoted as the C_3H-IICrgl mice, no mammary tumors ap-

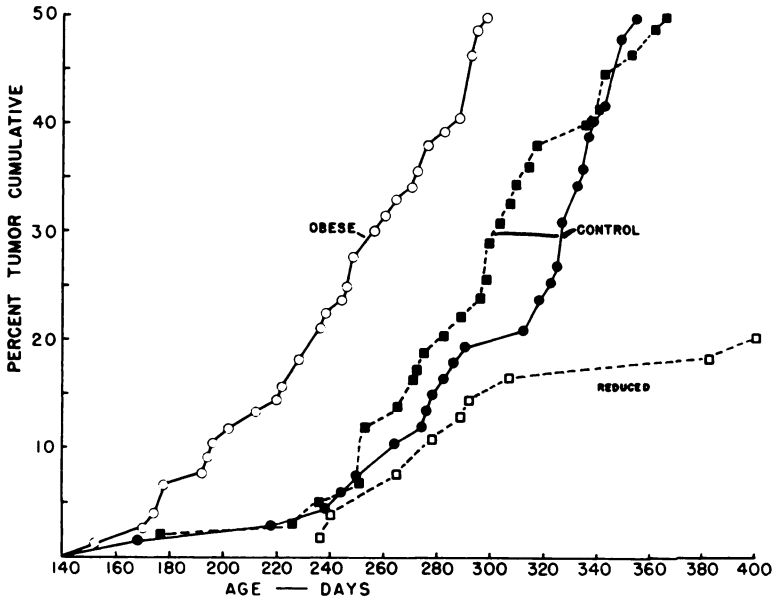

FIG. 4. Occurrence of spontaneous mammary tumors in obese, control and reduced (previously obese) virgin female C_3H mice.

peared during a period of twelve months. Similarly, during this same period C_3H-IICrgl mice made experimentally obese developed no mammary tumors. It was apparent that obesity and associated caloric intake do not incite the production of mammary tumors in animals lacking the specific tumor agent (Table 1). When the factor for tumorigenesis is present, the incidence can be increased or decreased by multiple factors from either within or without the animal. When the factor is missing, obesity or increased caloric intake, which otherwise accelerates or augments tumor appearance and incidence, has no effect at all.[14]

It has been demonstrated that tumor incidence in female C_3H mice is markedly reduced following gonadectomy. We believed that a study of the augmenting effect of obesity on tumor incidence as opposed to the depressing effect of castration would be valuable. A comparison of the tumor production of obese mice and mice of normal weights which had been castrated at the age of two months, with uncastrated normal and obese mice yielded typical results: the obese mice developed the greatest number of tumors or 46 per cent; the control animals, 31 per cent; the castrated obese mice, 14 per cent; and no tumors developed in the castrated mice of normal weights. Their ages at the onset of mammary tumors averaged 270

TABLE I

Incidence of Spontaneous Mammary Carcinoma in Female C_3H and C_3H-IICrgl Mice

Group of Mice	No. of Animals	Average Weight (gm.)	No. of Tumors	Per Cent of Tumors	Average Age at Tumor Onset (Days)
C_3H-IICrgl control	32	30	0	0	..
C_3H-IICrgl obese	30	52	0	0	..
C_3H control	23	32	8	35	333
C_3H obese	21	54	12	62	252

TABLE II

Effect of Castration on the Onset of Spontaneous Mammary Tumors in Female Obese C_3H Mice

Group of Mice	No. of Animals	Average Weight (gm.)	No. of Tumors	Per Cent of Tumors	Average Age at Tumor Onset (Days)
Control	35	32	11	31	298
Control-castrated	41	34	0	..	..
Obese	41	50	19	46	270
Obese-castrated	49	49	7	14	317

TABLE III

Incidence of Hepatomas in Obese, Control and Pair-Fed (Previously Obese) Male C_3H Mice

Group of Mice	No. of Animals	Average Weight (gm.)			Hepatomas	
		Initial	4 Weeks	13 Months	No. of Tumors	Per Cent of Tumors
Control	22	26	28	36	2	9.9
Obese	9	26	42	48	5	55.5
Pair-Fed	24	26	42	35	4	16.6

days in the obese mice, 298 days in the control animals and 317 days in the castrated obese mice. When this experiment was concluded at the end of one year, no tumors had appeared in the castrated control animals (Table II). The number of tumors which developed in the obese castrated group of mice was under the influence of two factors: (1) the removal of ovaries suppressed the appearance of tumors generally found in obese and control animals; (2) the increased caloric intake, obesity *per se*, augmented tumor development, and thus partially negated the influence of castration.

Continuing in a similar line of investigation we were able to show that spontaneously developing hepatomas increased in male C_3H mice made obese by administration of gold thioglucose. In a typical experiment, 64 per cent of the obese mice developed hepatomas; whereas, only 28 per cent of the control mice developed primary tumors of the liver.[15] The incidence of hepatomas in mice which were made obese and subsequently reduced to the weight of the control mice by paired feeding was close to that of the control animals. These reduced mice, therefore, did not develop the great increase of tumor incidence evident in obese mice which were allowed to eat *ad libitum*. It was apparent that having the potential of obesity did not lead to an unnatural occurrence of tumors (Table III). Only persistent obesity over a sufficient period of time was of importance.

TABLE IV

Incidence of Spontaneously Developing Hepatomas in Control, Obese and "Multiple Injected" (Cumulative) Male C_3H Mice

Group of Mice	No. of Animals	Average Weight (gm.)	Hepatomas	
			No. of Tumors	Per Cent of Tumors
Control	32	33	5	16
Obese	31	47	14	45
Cumulative	29	32	1	3.4

TABLE V

Incidence of Spontaneous Hepatomas in Control and Obese, Male and Female, C_3H-IICrgl Mice (Lacking Tumor Agent) at the End of Twelve Months

Group of Mice	No. of Animals	Average Weight (gm.)	No. of Tumors	Per Cent of Tumors
Male control	36	32	4	11
Male obese	29	50	10	34
Female control	32	30	1	3.1
Female obese	24	49	12	50

In all experiments, male animals which did not become obese following injection with a toxic dose of gold did not show an increase in incidence of liver tumors. Apparently the mere presence of gold itself was of no consequence. It was noted that a large amount of gold in the liver, induced by multiple small intraperitoneal injections of gold thioglucose (the total of which is many times greater than the single toxic dose) was not a factor in the increased incidence of hepatomas. A single dose sufficient to produce obesity resulted in increased incidence of neoplasia (Table IV).

Generally, it has been difficult to ascertain the number of hepatomas in female C_3H mice for usually death due to mammary tumors occurs and the animals do not survive long enough to give a true incidence of the hepatomas which would occur.[16] The use of C_3H-IICrgl mice lacking the mammary tumor factor circumvented this obstacle. The appearance of a single hepatoma in our female group at the end of one year agrees with literature wherein the reported incidence of hepatomas in females is small and much lower than in males. However, when the females were allowed to become obese there was a definite increase in the occurrence of liver tumors. At the end of twelve months 50 per cent of our female mice had such tumors (Table V).

We have observed no pregnancies in the truly fat C_3H mice during the ten years of producing obesity by the administration of gold thioglucose. In an effort to study the factors associated with this observation we paired female C_3H mice, which were four months old, with normal males; within the next month all of the females were pregnant. After delivery and weaning, the mice were put into three groups: one group in which the parous mice received a single dose of 8 mg. of gold thioglucose; one in which the mice were given multiple small doses for thirty days; and one group in which the mice remained untreated. Six weeks later the animals of these groups were again mated with normal males. At that time the obese mice averaged 48 gm. in weight, the mice giving multiple doses weighed an average of 29 gm. and the average weight of the control group was 31 gm. No pregnancies appeared in the group of obese mice while all the animals in the "multiple injected" and control groups became pregnant. Subsequently, the obese mice, starved down by paired feeding to the weight of the control animals (i.e., about 32 to 34 gm.), were again allowed contact with males. No pregnancies appeared in this group of previously-obese reduced mice.

As a continuation of our work concerning pregnancies in obese animals, smears of C_3H virgin female mice were studied over a period of time. After a set pattern of estrus cycle was determined, a certain number of these animals were made obese. A change in the cytology of the vagina was apparent in subsequent smears of these obese animals. In most of the animals long periods of cornification of cells had occurred. When such animals were mated with males known to be capable of impregnation, no offspring were produced. None of the animals which became what we considered truly obese produced offspring; however, litters were produced by those gold-treated animals which did not become obese.

The ovaries from obese mice, in which long periods of cornification of cells had occurred, were observed (macro- and microscopically) and found to be atrophic. They consisted of follicles and only a few old corpora lutea. This was indicative of a continuous estrogenic phase, which may have accounted for the augmentation of at least the mammary tumors in obese mice.

SUMMARY

In summary, we have shown that mice can be made obese by injection of gold thioglucose. Animals so treated develop lesions in the hypothalamus and subsequently become obese. Animals made obese by this technic show two interesting phenomena: such mice have an augmentation of tumor production and truly obese animals do not become pregnant.

Hormonal imbalance occurs when hypothalamic obesity is produced. A condition results which apparently allows sufficient estrogen production to induce an increase in tumor production and at the same time keeps these obese animals from going through a normal estrus cycle.

REFERENCES

1. Brecher, G. and Waxler, S. H. Obesity in albino mice due to single injections of gold thioglucose. *Proc. Soc. Exper. Biol. & Med.*, 70: 498, 1949.
2. Waxler, S. H. and Enger, M. Organ weights and obesity in mice. *J. Nutrition*, 54: 209, 1954.
3. Waxler, S. H. and Brecher, G. Obesity and food requirements in albino mice following administration of gold thioglucose. *Am. J. Physiol.*, 162: 428, 1950.
4. Andervont, H. B. Spontaneous tumors in a subline of strain C_3H mice. *J. Nat. Cancer Inst.*, 1: 737, 1941.
5. Baumann, C. A., Jacobi, H P. and Rusch, H. P. The effect of diet on experimental tumor production. *Am. J. Hyg.*, Sect. A, 30: 1, 1939.
6. Boutwell, R. K., Brush, M. K. and Rusch, H. P. The stimulating effect of dietary fat on carcinogenesis. Cancer Res., 9: 741, 1949.
7. Jacobi, H. P. and Baumann, C. A. The effect of fat on tumor formation. *Am. J. Cancer*, 39: 338, 1940.
8. Rusch, H. P. Extrinsic factors that influence carcinogenesis. *Physiol. Rev.*, 24: 177, 1944.
9. Tannenbaum, A. The initiation and growth of tumors. I. Effects of underfeeding. *Am. J. Cancer*, 38: 335, 1940.
10. Visscher, B., Ball, Z. B., Barnes, R. H. and Sivertsen, I. The influence of caloric restriction upon the incidence of spontaneous mammary carcinoma in mice. *Surgery*, 11: 48, 1942.
11. Waxler, S. H., Tabar, P. and Melcher, L. R. Obesity and the time of appearance of spontane-

ous mammary carcinoma in C_3H mice. *Cancer Res.*, 13: 276, 1953.

12. WAXLER, S. H. The effect of weight reduction on the occurrence of spontaneous mammary tumors in mice. *J. Nat. Cancer Inst.*, 14: 1253, 1954.
13. BITTNER, J. J. Some possible effects of nursing on the mammary gland tumor incidence in mice. *Science*, 84: 162, 1936.
14. WAXLER, S. H. The relationship of obesity to cancer production in C_3H-IICrgl mice (lacking in mammary tumor agent). *Stanford M. Bull.*, 15: 26, 1957.
15. WAXLER, S. H. and TABAR, P. Appearance of hepatomas in obese C_3H male mice. *Stanford M. Bull.*, 11: 272, 1953.
16. ANDERVONT, H. B. Studies on the occurrence of spontaneous hepatomas in mice of strains C_3H and CBA. *J. Nat. Cancer Inst.*, 11: 581, 1950.
17. WAXLER, S. H. and TABAR, P. The relationship of obesity to liver tumors in mice. *Stanford M. Bull.*, 18: 1, 1960.

DISCUSSION

DR. JEAN MAYER (*Boston, Massachusetts*): Without wanting to detract from the results that have been presented here, I think that we should guard against the tendency to generalize results unduly. Dr. Waxler has not done it. But I think there would be a temptation to conclude that there is a promotion of tumors by a high caloric intake.

If we look at a table of mortality in relation to body weight in man we see that there are a great many diseases in which mortality is increased by obesity: diabetes, heart disease, et cetera. There are two causes of death which are decreased: tuberculosis and suicide. The deaths from tumors are the same in obese persons as in non-obese persons.

I do not think that this means that there is no interaction between caloric intake and tumors, but I think that it may depend on the type of tumors and the type of obesity.

A few years ago we demonstrated that in the obese hyperglycemic syndrome there is an increased resistance to liver ascites tumors, which is an unusual type of tumor, and an unusual type of obesity. I think this illustrates the fact that the interaction may be extremely complex.

One point I was interested in was the fact that, Dr. Waxler and his co-workers have had no luck in obese pregnant gold thioglucose animals, which we have observed and which have always been observed. There is a paper by Hamilton in this subject, which reported a number of obese pregnant animals. And there was also a French paper a few years ago, I have forgotten the authors, which described a number of obese gold thioglucose animals which became pregnant.

I was wondering whether the duration of the obesity might be a factor in the ability of these animals to become pregnant.

DR. WAXLER: No, I do not think that is the factor We have studied the reactions of these obese mice, in various strains, for about twelve years. An instillation of sperm can be performed, but this does not result in pregnancy. We have used young and old males, and young and old females in these experiments.

We thought there might be even physiologic difference between these animals, but we have not proven this. The animals which we used were genetically pure, and usually produce the first time they are mated. This pattern of reproduction ceases when they are made obese. Smears indicate that they are sterile. If the animals have a continuous estrogenic phase, with cornification of cells, and they do not show any corpora lutea and if what one sees are the old corpora lutea which may have been there before the mice were injected, then I cannot see how pregnancy could be induced.

DR. JAY TEPPERMAN (*Syracuse, New York*): I agree with Dr. Mayer that you really cannot learn very much by looking at over-all cancer statistics in obesity.

Some years ago, stimulated by Dr. Waxler's publications, we investigated some of the clinical reports on the incidence of cancer in obese patients. We noted that there was some indication in the clinical literature that the incidence of both cancer of the breast and endometrial cancer concurred with obesity whereas other types of cancer did not. There was a clear-cut association between the incidence of obesity and incidence of cancer in these two types of cancer—the same two types studied by Dr. Waxler.

DR. C. N. H. LONG (*New Haven, Connecticut*): We also noted the failure of the reproductive function in the females of these animals. There was a reduction in the size of the ovary in the rats which we studied. At that time, we were inclined to associate this with the development of obesity. Now, however, I believe that these failures are probably due to damage to other areas of the hypothalamus that are concerned with the release of the ovulation hormone.

DR. Waxler, did you observe the testicular function in the obese male mice?

DR. WAXLER: No, we did not.

DR. B. K. ANAND (*New Delhi, India*): I agree with Dr. Long's remarks. If the obesity has been produced by giving gold thioglucose, there will be damage to large areas in the hypothalamus. From experimental work, it is evident that the regulation of the gonadotropic function from the hypothalamus, at least in albino rats, is in the area surrounding the vendomedial nucleus. If some other means of producing obesity is used, these disturbances in the hormonal patterns may not be evident. But if you use large doses of gold thioglucose and damage this area extensively, not only the gonadotropin-secreting activity but other hormonal patterns will be upset.

Therefore, is it the simple increase in food intake which influences the production of cancer or of these tumors? Is it the disturbances of all these hormonal patterns in these animals? If this problem is studied

again without producing damage to this area of the hypothalamus, but by producing obesity by force feeding or giving high caloric diets, it will be interesting to see if there is a change in the tumors then.

DR. WAXLER: There is an increase in tumors in those animals that have been confined in very small areas. They do not get quite as large as the animals that are given gold. Of course, these confined animals are able to reproduce.

DR. TEPPERMAN: When you were studying hepatomas in restricted-feeding situations in animals with hypothalamic lesions, there seemed to be a slight suggestion that in those animals the incidence of hepatomas was slightly greater than in the control group. I wonder whether those animals ate their food all in one gulp, whether they were really meal-eaters, as Dr. Cohn has taught us.

DR. WAXLER: I do not know.

DR. ESTELLE R. RAMEY (*Washington, D. C.*): Dr. Anand's suggestion is ruled out by Dr. Waxler's experiments in which he fasted the animals with similarly large lesions. In those instances one would anticipate the same degree of endocrine anomalies, and yet those animals, in some instances, had fewer tumors.