Studies of the Effect of Perthane on Spontaneous and Transplanted Mammary Carcinoma in the C3H Mouse*

LOUIS A. LEONE, HENRY B. HAHN, JR., AND SUSAN J. MELLETTE

(Division of Cancer Studies and the Department of Medicine, Medical College of Virginia, Richmond, Va.)

DDD—2,2-bis-(para-chlorophenyl)-1,1-dichloroethane—a derivative of DDT—2,2-bis-(para-chlorophenyl)-1,1,1-trichloroethane—causes severe adrenal cortical atrophy in the dog (9, 15–17, 25). Larson *et al.* (9) found that the two chlorines in the 1-position of the ethane group were critical, with adrenal cortical atrophy failing to develop with the chlorine at the 3 or 1 position, or absent (Chart 1). These workers also demonstrated that, while the chlorines in the para-phenyl position are not essential, the phenyl rings must be present. Substitution of ethyl radicals for the chlorines in the para-phenyl position of DDD results in the formation of 2,2-bis-(para-ethylphenyl)-1,1-dichloroethane (Perthane)[1] (Chart 1). Perthane has the same adreno-corticolytic effect in the dog as DDD (9).

Shimkin and Wyman (19) have demonstrated that adrenalectomy in unbred C3H female mice of the Andervont strain, at the age of 4 weeks, decreases the incidence of spontaneous mammary carcinoma from 84.5 to 40 per cent. Martinez and Bittner (13) reported a 3.3 per cent incidence of complete regression and a 10 per cent incidence of partial regression of mammary tumors in mice after total bilateral adrenalectomy when the spontaneous tumors were 5–14 mm. in diameter. Although histologic evidence of adrenocortical atrophy has not been reported in Perthane-fed mice, the possibility that physiological depression might occur has not been excluded by previous work. This consideration suggested to us that greater adrenal hypofunction might result from prolonged Perthane administration and, furthermore, might be reflected specifically by a decrease in the formation of spontaneous mammary carcinoma. In addition to studies of spontaneous tumors, experiments on transplanted mammary tumor were carried out to observe any possible direct anti-tumor effect of Perthane.

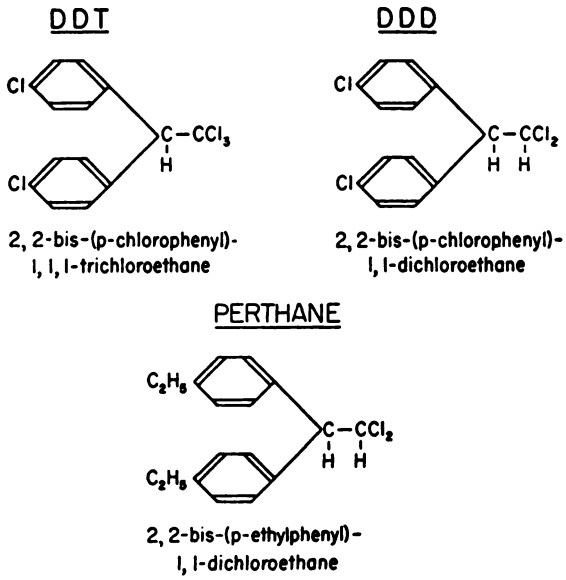

CHART 1.—Structure of Rhothane (DDD) and derivatives

MATERIALS AND METHODS

Spontaneous tumor studies.—Unbred females of the C3H/Jax strain were used. All animals were obtained from the Jackson Memorial Laboratory, Bar Harbor, Maine, in a single lot and had been born within the same 2-week period. The experiment was begun when the animals were 6–7 weeks of age. They were housed in clear plastic cages (11×11×6½) of ten animals each and were fed ground Purina chow and water ad libitum.

The mice were divided into six groups, as indicated in Table 1. The dosage of Perthane was calculated on the basis of the initial average weight of each group in which the weights did not vary more than 1 gm. among individual animals. The oral 24-hour LD_{50} for dry Perthane intimately mixed with food had

* This research was supported in part by grant CT 496 of the National Cancer Institute of the National Institutes of Health, Public Health Service; and the facility of the Samis Grotto Cancer Research Laboratory, Medical College of Virginia.

[1] Perthane was generously supplied by Rohm and Haas Company of Philadelphia.

Received for publication March 19, 1958.

been previously determined for female mice of this strain in our laboratories and was found to be 9,340 mg/kg/24 hours. The maximum sublethal 20-day oral dose of Perthane mixed with ground chow was found to be 0.5 × LD_{50} per day. Doses exceeding this level resulted in death for the majority of the mice in less than 10 days.

The animals in Groups I–IV were administered Perthane for life as indicated in Table 1. Animals in Groups V and VI received higher initial doses to determine whether early high Perthane levels would result in greater tumor inhibition than in Group III (0.2 × LD_{50} for life).

Perthane for oral administration was prepared by being dissolved in acetone and by a thorough wetting of the ground Purina chow. The acetone was permitted to volatilize completely, and feeding was begun 24 hours later. The fraction of the LD_{50} dose was determined by the initial weight and was not altered thereafter. Inasmuch as Perthane is stored in great quantities in body fat, it was considered that the upward adjustment of dosag with increasing animal weight would result in excessive toxicity. The animals were weighed and examined daily during the initial 30 days and once weekly thereafter.

Transplanted tumor studies.—C3H/Jax males, 6–7 weeks of age and housed in cages (11×11×6¼) of ten animals each, were used. BA mammary carcinoma obtained from Jackson Memorial Laboratory and maintained by trocar transfers to several C3H animals at 18–21-day intervals (10 mm. tumor size) were used in this work. Transplants (2-mm. size) were introduced subcutaneously in the right lateral abdominal area by a trocar method. Animals were nearly uniform in weight, with a variation of from 1 to 2 gm. among individuals. Animal weight and tumor size were recorded daily. Tumors were measured in their greatest single diameter with a special caliper (Buffalo Dental Manufacturing Company). Ground Purina chow was fed ad libitum, and Perthane-dosed feed was prepared by the volatilization technic described under "Spontaneous tumor studies."

The animals were divided into four groups: absolute controls, tumor controls, and Perthane controls—each containing ten animals; and the fourth group, containing twenty animals, received both the tumor and Perthane. This general plan was used to study: (*a*) effect of 10- and 20-day courses of Perthane started at time of tumor transfer; and (*b*) effect of a 20-day course of Perthane started when the tumor measured 10 mm. in diameter. The maximum sub-lethal dose of 0.5 × LD_{50} × 10 days, and 0.45 × LD_{50} × 20 days produced animal toxicity in two to three animals in the treated groups on the last day of administration. No deaths resulted from Perthane dosage.

In studies of both spontaneous and transplanted tumors, histologic confirmation of tumors was made at time of autopsy. A few animals in tumor-bearing groups died unobserved as a result of our attempt to permit accumulation of accurate data on survival time.

RESULTS

Spontaneous tumor growth.—The spontaneous tumor incidence and survival time are given in Table 1. Accidental death accounted for a loss of from one to three animals in Groups II–VI and occurred prior to the appearance of the first tumor on day 253 in a Group II animal. The control Group I and Group II developed tumors in 80 and 89 per cent of instances, respectively. This is an incidence expected in unbred females of this strain. It is of interest that the ratio of the total number of tumors to the number of animals with tumors is distinctly higher in control Group I than in the minimally dosed Group II. There is no appreciable difference in first tumor incidence between Groups I and II. A definite inhibition of tumor formation is present in animals receiving 0.2 × LD_{50} per day (Group III). At the dose of 0.3 × LD_{50} per day in Group IV (Table 1), no spontaneous mammary tumors were noted. One animal in this group was found to have an ulceration in the abdominal wall which was proved histologically to be a subcutaneous sarcoma. This tumor was transferred successfully to healthy C3H hosts and did not resemble mammary carcinoma in any way.

A group containing 25 animals and receiving 0.3 × LD_{50} per day is being studied currently with suitable controls, and after 540 days of Perthane administration none of the treated animals has developed spontaneous mammary carcinoma. The

TABLE 1

THE EFFECT OF PERTHANE ON THE INCIDENCE OF SPONTANEOUS MAMMARY CARCINOMA AND SURVIVAL TIME IN THE C3H MOUSE

Group	Dose* ×LD_{50}/day	Duration	Animals, total no.†	Animals with tumors: No. with mammary tumors	No. of tumors	Latent period (days)	Av. survival (days)	Animals without tumors: No. without tumors	Av. survival (days)	Combined Av. survival (days)
I (control)	0	Life	10	8	16	410	460	2	512	471
II	0.1	Life	18	16	20	373	437	2	477	413
III	0.2	Life	18	10	12	416	468	8	543	501
IV	0.3	Life	19	0	1‡	0	579	18	553	555
V	0.4	20 days	18	10	12	452	514	8	571	539
	0.3	10 days								
	0.2	Life								
VI	0.5	20 days	17	8	8	427	489	9	572	533
	0.4	10 days								
	0.2	Life								

* Oral LD_{50} = 9,340 mg/kg body weight.

† Twenty animals were started in all groups except Group I, in which ten animals were used.

‡ One animal in Group IV was found to have a subcutaneous sarcoma.

control group has demonstrated eighteen tumors to date.

The incidence and total number of tumors in Groups V and VI are comparable to those in Group III. The ultimate effective dose in these groups, therefore, is 0.2 × LD_{50} per day. It appears that initial high dosage under the conditions of this experiment does not affect spontaneous tumor formation. The maximum sub-lethal chronic dose of 0.3 × LD_{50} per day is required to inhibit tumor formation completely.

The interval prior to the appearance of spontaneous tumor in Perthane-treated animals (except in Group IV, in which no mammary carcinoma was seen) varies greatly. No significant delay in the average time of onset of the spontaneous tumor formation was noted in Perthane-treated animals, except in Group IV where no breast tumors occurred. Survival time does not appear to be greatly influenced by Perthane administration apart from its effect on tumor incidence. Survival time of tumor-bearing and tumor-free animals receiving no Perthane (Group I) and that of those receiving 0.3 × LD_{50} per day (Group IV) are significantly different. However, the greater average survival time of the animals in Group IV (555 days) reflects the absence of spontaneous tumor occurrence in this group. The two animals in Group I which did not develop tumors had an average survival time of 512 days. Group I animals generally experienced a high tumor incidence and a resultant lower survival period. Tumor-free animals receiving 0.2 × LD_{50} per day of Perthane (Groups III, V, and VI) had average survival rates comparable to that of Group IV. Their combined average survival, however, was slightly lower than that of the optimally treated group (Group IV). Therefore, animals not developing tumors demonstrated no increased survival rates purely as a result of receiving Perthane.

The initial weight loss induced by Perthane stabilized during long-term treatment. Mice receiving 0.3 × LD_{50} per day weighed approximately 13 per cent less than untreated animals up to the time the first tumor was observed. At dosage levels of 0.1 and 0.2 × LD_{50} per day (Groups II, III, V, and VI), Perthane-treated animals weighed from approximately 4 to 8 per cent less than the control group. The cause of this weight difference has not been determined. Special feeding experiments using five animals each from the control Group I and the optimal treatment Group IV were carried out in metabolic cages. These animals were observed during a 16-day period beginning on day 418 of the experiment. Tumors had not yet appeared in the control animals. The average daily food intake per mouse in the treated group was 3.22 gm., and in the control group 3.49 gm. This represents a difference of 8 per cent in total daily food intake.

The effect of reduced dietary intake on the formation of spontaneous mammary carcinoma in mice has been reported (22–24). White *et al.* (24) demonstrated that a caloric reduction of 50 per cent in virgin C3H mice reduced spontaneous tumor incidence from 100 to 12.5 per cent. Mice on a restricted diet weighed an average of 13.7 gm. and had a nose-to-anus length of 8.9 cm. Animals fed a normal caloric diet weighed an average of 30.7 gm.

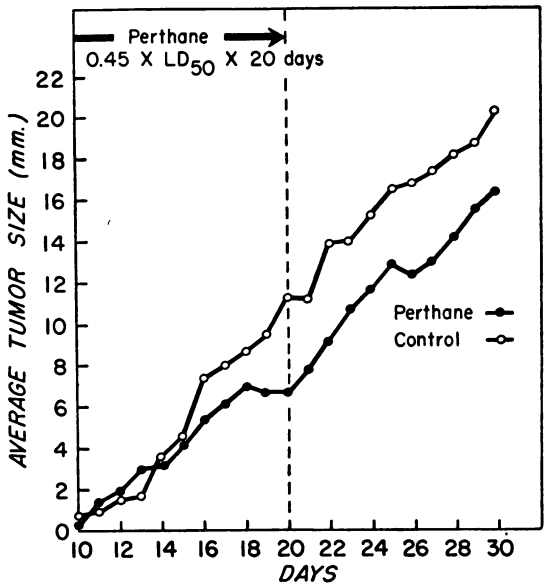

CHART 2.—Effect of Perthane on tumor size; 20-day drug administration was begun when tumor was implanted.

and had a body length of 11.0 cm. (24). In the present study, body length was approximately the same in all groups. The weight differences noted in the present study are not considered to have a significant effect on spontaneous tumor incidence. Animals fed Perthane over an extended period, as compared with control mice, demonstrate body fat depletion which may account for the decrease in body weight.

Transplanted tumors.—When Perthane was instituted at the time of tumor transfer and continued for 10 days, no inhibition of tumor growth occurred. When treatment was continued for a period of 20 days (Chart 2), a brief delay in the rate of tumor growth was noted between days 16 and 20 of Perthane administration. This change in rate of growth was only temporary with subsequent growth rate of treated tumor running a course parallel to that of the controls after discontinua-

tion of Perthane. To rule out the possibility that the newly transplanted tumor was damaged during the first 14–16 days of Perthane treatment, Perthane effect on the 10-mm. tumor was studied (Chart 3). It was considered that, if Perthane were effective, it would inhibit the rate of growth of a healthy, well established, small transplant. It is apparent from the diagram that no such effect occurred, with the growth rate in treated and untreated groups almost identical. No significant difference in survival time occurred between the treated and untreated groups (Chart 4).

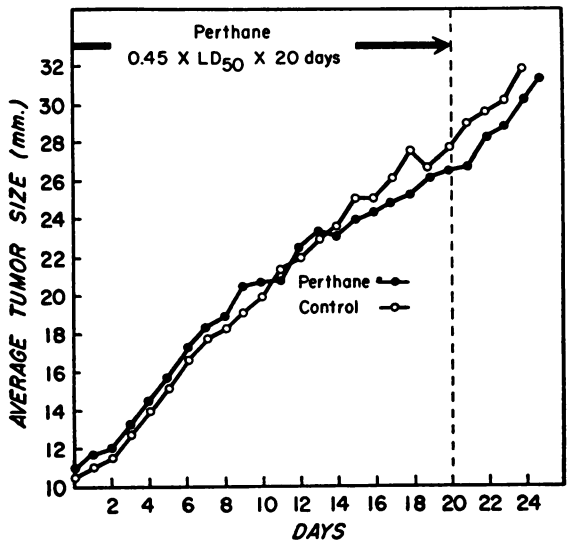

CHART 3.—Effect of Perthane on tumor size; 20-day drug administration was begun when tumor was 10 mm. in diameter.

DISCUSSION

The results of this study indicate that Perthane has a potent inhibitory effect upon the spontaneous occurrence of mammary carcinoma in the unbred C3H female mouse. Decreased caloric intake does not appear to be a significant factor in the as yet undefined mechanism of this effect. Weights of animals fed Perthane remained at a level well above that required to influence tumor incidence or growth. Survival was not affected materially. Experiments by Finnegan *et al.* (6) indicated similar weight changes and unaltered survival in the rat.

The ability of Perthane to prevent spontaneous mammary tumor formation appears to be related to its influence on the endocrine system. In all treated animals the ovaries were reduced in size, and luteal tissue was greatly decreased on histologic examination. Uterine and vaginal epithelial atrophy were accompanied by vaginal cytologic evidence of impairment of the estrous cycle in treated animals. The adrenal cortex in treated mice demonstrated no significant histological changes, and adrenal weights were not altered from the normal.

Physiologic activity of the adrenal cortex was not evaluated in this study; however, adrenal cortical dysfunction has been reported by Brown in rats treated with DDD (3).

Bleiberg has indicated that, although adrenal cortical atrophy does not occur in Perthane-fed rats, ACTH-stimulated adrenal weight increase could be partially inhibited by Perthane (2). Cobey *et al.*, working with dogs (4), and Taliaferro and Leone, who studied humans (21), report a depression of plasma 17-hydroxycorticosteroid levels following ACTH stimulation and Perthane administration. The failure to observe histological changes in the adrenal cortex in the present study does not preclude the presence of some undetermined physiologic effect.

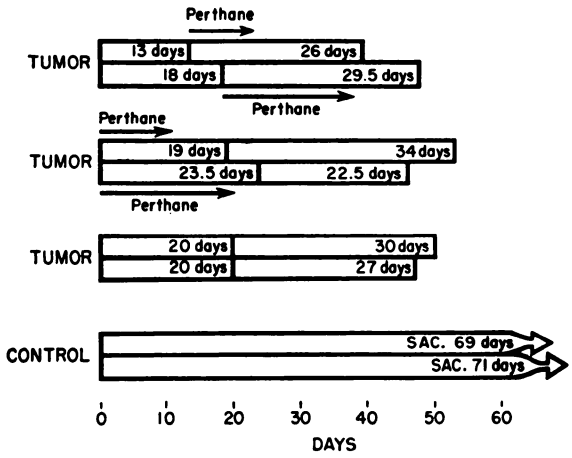

CHART 4.—Survival time of animals with transplanted tumor. The first segment in each bar represents time required for tumor to reach 10 mm. The arrow indicates 10 or 20-day course of Perthane administration.

The effect of ovariectomy in lowering the incidence of spontaneous mammary carcinoma has been reported by several authors (5, 10, 11, 14, 19, 20, 26). More recently, King *et al.* (8) and Pilgrim (18) have suggested that the formation of spontaneous mammary carcinoma may be unaffected by ovariectomy. Pilgrim (18) describes a method which carefully considers the influence of accidental death in the interpretation of results. It is likely that adrenal secretion of estrogens stimulates delayed mammary carcinoma formation in the ovariectomized C3H mouse (12, 18). The complete inhibition of spontaneous mammary tumor formation in this study may be explained by the inability of the adrenal cortex in the presence of Per-

thane to respond to the relative decrease in ovarian activity. Studies being carried out in ovariectomized mice may supply further information regarding the ability of the adrenal cortex to substitute for ovarian estrogen in the presence of Perthane administration.

Although it appears that Perthane may prevent spontaneous mammary carcinoma by interfering with estrogen formation by the ovary and adrenal cortex, the fact that ovarian atrophy is not more nearly complete and luteinization is primarily affected suggests that some estrogen is formed. In this event, Perthane could act as a metabolic "blocking agent" preventing estrogen stimulation of uterus, breast, and vaginal epithelium. This mechanism could also account for the failure of the adrenal cortex to hypertrophy inasmuch as trophic stimulation from the pituitary would not be induced.

Finally, the effect of Perthane on pituitary function requires further investigation. In hypophysectomized female mice injected with estrogen, mammary glands fail to develop (1, 7). The possibility that Perthane inhibits some "mammotrophic" substance must be considered. This may be of particular importance in view of the complete depression of the formation of spontaneous breast carcinoma observed in this study.

SUMMARY

1. Perthane, fed to C3H unbred female mice in a maximum tolerated dosage for life, prevented the formation of spontaneous mammary carcinoma in all animals treated.

2. No direct anti-tumor effect could be demonstrated in studies of transplanted mammary carcinoma in the C3H mouse.

3. Decreased ovarian size, depressed corpus luteum activity, as well as uterine and vaginal epithelial atrophy occurred in animals treated for long periods. No evidence of adrenal cortical hypoplasia or hyperplasia could be demonstrated histologically in animals receiving Perthane in our experiments.

4. Possible mechanisms of the action of Perthane are discussed.

ACKNOWLEDGMENTS

The technical assistance of Mrs. Roberta O'Keefe is gratefully acknowledged.

REFERENCES

1. ALLEN, E.; DANFORTH, C. H.; and DOISY, E. A. Sex and Internal Secretions. 2d ed. Baltimore: Williams & Wilkins, 1939.
2. BLEIBERG, M. J., and LARSON, P. S. Studies on Derivatives of 2,2-bis-(*p*-chlorophenyl)-1,1-dichloroethane (DDD, TDE) with Special Reference to Their Effects on the Adrenal Cortex. J. Pharmacol. & Exper. Therap., **121**:421–31, 1957.
3. BROWN, J. H. U. Influence of the Drug DDD on Adrenal Cortical Function in Adult Rats. Proc. Soc. Exper. Biol. & Med., **83**:59, 1953.
4. COBEY, F. A.; TALIAFERRO, I.; and HAAG, H. B. Effect of DDD and Some of Its Derivatives on Plasma 17-OH Corticosteroids in the Dog. Science, **123**:140, 1956.
5. CORI, C. F. The Influence of Ovariectomy on the Spontaneous Occurrence of Mammary Carcinoma in Mice. J. Exper. Med., **45**:983–91, 1927.
6. FINNEGAN, J. K.; HENNIGAR, G. R.; SMITH, R. B., JR.; LARSON, P. S.; and HAAG, H. B. Acute and Chronic Toxicity Studies on 2,2-bis-(*p*-ethylphenyl)-1,1-dichloroethane (Perthane). Arch. Int. Pharmacodyn., **53**:404–18, 1935.
7. GARDNER, W. U. Growth of the Mammary Glands in Hypophysectomized Mice. Proc. Soc. Exper. Biol. & Med., **45**:835, 1940.
8. KING, J. T.; CASAS, C. E.; and VISSCHER, M. B. The Influence of Estrogen on Cancer Incidence and Adrenal Changes in Ovariectomized Mice on Caloric Restriction. Cancer Research, **9**:436–37, 1949.
9. LARSON, P. S.; HENNIGAR, G. R.; FINNEGAN, J. K.; SMITH, R. B., JR.; and HAAG, H. B. Observations on Relation of Chemical Structure to Production of Adrenal Cortical Atrophy or Hypertrophy in Dog by Derivatives of 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethane (DDD,TDE). J. Pharmacol. & Exper. Therap., **115**:408–12, 1955.
10. LATHROP, A. E. C., and LOEB, L. Further Investigations on the Origin of Tumors in Mice. III. On the Part Played by Internal Secretions in the Spontaneous Development of Tumors. J. Cancer Research, **1**:1–19, 1916.
11. LOEB, L. Further Investigations on the Origin of Tumors in Mice. VI. Internal Secretion as a Factor in the Origin of Tumors. J. Med. Research, **40**:477–96, 1919.
12. MARTINEZ, C., and BITTNER, J. J. Postcastrational Adrenal Tumors in Unilaterally Adrenalectomized C3H Mice. Cancer Research, **15**:612–13. 1955.
13. MARTINEZ, C., and BITTNER, J. J. Effect of Ovariectomy, Adrenalectomy and Hypophysectomy on Growth of Spontaneous Mammary Tumors in Mice. Proc. Soc. Exper. Biol. & Med., **86**:92, 1954.
14. MURRAY, W. S. Ovarian Secretion and Tumor Incidence. Science, **66**:600–601, 1927.
15. NELSON, A. A., and WOODARD, G. Severe Adrenal Cortical Atrophy (Cytotoxic) and Hepatic Damage Produced in Dogs by Feeding 2,2-bis(para-chlorophenyl)-1,1-dichloroethane (DDD,TDE). Arch. Path., **48**:387–94, 1949.
16. NICHOLS, J., and GARDNER, L. I. Production of Insulin Sensitivity with Adrenocorticolytic Drug DDD (2,2-bis-(para-chlorophenyl)-1,1-dichloroethane). J. Lab. & Clin. Med., **37**:229–38, 1951.
17. NICHOLS, J., and SHEENAN, H. L. Effect of Partical Adrenal Cortical Atrophy on Course of Alloxan Diabetes. Endocrinology, **51**:362–77, 1952.
18. PILGRIM, H. I. A Method of Evaluating Tumor Morbidity as Applied to the Effect of Ovariectomy at Different Ages on the Development of Mammary Tumors in C3H Mice. Cancer Research, **17**:405–8, 1957.
19. SHIMKIN, M. B., and WYMAN, R. S. Effect of Adrenalectomy and Ovariectomy on Mammary Carcinogenesis in Strain C3H Mice. J. Nat. Cancer Inst., **6**:187–89, 1945.
20. SMITH, F. W. The Relationship of the Inherited Hormonal Influence to the Production of Adrenal Cortical Tumors by Castration. Cancer Research, **8**:641–51, 1948.
21. TALIAFERRO, I., and LEONE, L. Inhibitory Effect of Perthane (2,2-bis-(para-ethylphenyl)-1,1-dichloroethane) on

Adrenocortical Function in Human Subjects. New Eng. J. Med., **257**:855–60, 1957.

22. Tannenbaum, A. Dependence of Tumor Formation on the Degree of Caloric Restriction. Cancer Research, **5**:609–15, 1945.
23. Visscher, M. B.; Ball, Z. B.; Barnes, R. H.; and Sivertson, I. Influence of Caloric Restriction upon the Incidence of Spontaneous Mammary Carcinoma in Mice. Surgery, **11**:48–55, 1942.
24. White, F. R.; White, J.; Mider, J. B.; Kelly, M. G.; Heston, W. E.; and David, P. W. Effect of Caloric Restriction on Mammary Tumor Formation in Strain C_3H Mice on the Response of Strain dba to Painting with Methylcholanthrene. J. Nat. Cancer Inst., **5**:43–48, 1944.
25. Woodard, G.; Davidow, B.; and Nelson, A. A. Effects Observed in Dogs Following the Prolonged Feeding of DDT and Its Analogs. Fed. Proc., **7**:266, 1948.
26. Woolley, G. W.; Fekete, E.; and Little, C. C. Tumor Development in Mice Ovariectomized at Birth. Proc. Nat. Acad. Sc., **25**:277–79, 1939.