The Production of a Carcinogenic Agent in the Degradation of Cholesterol to Progesterone*

Fritz Bischoff, Ph.D., and J. Jerome Rupp, Captain, M.C., U.S.N.R.

(From the Chemical Laboratory, Santa Barbara Cottage Hospital Research Institute, Santa Barbara, California)

(Received for publication February 5, 1946)

INTRODUCTION

Ever since the carcinogenic hydrocarbons and the steroid hormones, which may influence carcinogenesis, have been shown to be structurally related to cholesterol, the animal sterol par excellence, speculation has been active concerning both the endogenous and exogenous conversion of these compounds, one to another, as a possible cause of cancer (7). By means of degradative oxidation (and in some cases reduction) cholesterol may be converted to the steroid hormones in the test tube, and cyclization of the aliphatic side chain could give rise to the ring skeleton of the carcinogenic hydrocarbons. Carcinogens have been isolated from the liver (5) and cholesterol changed structurally by heat has been held responsible for gastric carcinoma (11). Although literally hundreds of steroid compounds have been prepared by the organic chemist, there are still wide gaps in the series of probable compounds that could form as intermediary steps in the path of a degradation. The possibility of these compounds serving as carcinogens or being more readily converted endogenously to carcinogens is not known to have been realized.

The present report is an outgrowth of an accidental discovery, which is considered a valuable clue to the ultimate solution of the problem.

In 1941, when this problem was begun, it was known that progesterone, like the estrogens, is intimately connected with the mechanism regulating the development and maintenance of the mammary gland; however, in contrast to the estrogens its influence on carcinogenesis had not been proved (1). Since the Marsh-Buffalo strain of mice is a high cancer strain resistant to the influence of estrogens (2), an investigation of the possible role of progesterone in carcinogenesis in this strain was prompted. A chemically crude but accurately standardized synthetic preparation was used in the initial experiments, and it proved to have carcinogenic properties. It was then necessary to repeat the experiment with crystalline progesterone, which was the biologically active ingredient in the original experiment; crystalline cholesterol, which was the starting material; and crystalline cholestenone, which was a known contaminating substance. These all proved to be noncarcinogenic. One had therefore the choice in pursuing this problem farther either of attempting to isolate the carcinogenic compound from the reaction product or of testing out known compounds that would be expected to form as links in the degradative chain on the basis of the chemical procedure. Both lines of attack are being pursued in this laboratory. Our original experiments are presented, in view of the rapid strides being made in steroid chemistry and the possibility that a compound that would fit into the chemical scheme yielding the carcinogenic effect might be isolated by some other investigator. Interest is also attached to the original purpose of the first experiment; namely, the role of progesterone in the development of mammary cancer.

EXPERIMENTAL

Plan of animal experimentation.—Virgin Marsh-Buffalo female mice were employed in 3 groups of long-term experiments. Each group contained a division of intact control mice, which received injections of sesame oil equal to the amount serving as a vehicle for the steroid given the other 2 divisions of the group. The mice of 1 division receiving the steroid were castrated at 23 days of age; those of the other remained intact. The injections in all cases were dorsal, and were given subcutaneously as far as possible from the mammary glands. Details of dosage follow.

GROUP I

Experiment 1.—Thirty-eight control mice received 0.08 cc. of sesame oil per mouse weekly; a total of 1.7 cc. per mouse was administered from the second to the ninth month of age.

Experiment 2.—Thirty-eight intact mice received a total of 10 units of crude progesterone per mouse.

Experiment 3.—Thirty-eight castrated mice received a total of 10 units of crude progesterone per mouse. (A biologic unit is 1 mgm., so that each mouse received in addition about 20 mgm. of supposedly inert material.)

* Aided by grants from The International Cancer Research Foundation, and from Mr. and Mrs. Herman F. Pelphrey. Presented at the Second Mexican Congress of Cancer in Guadalajara, Mexico, February 3, 1946.

Group II

Experiment 4.—Thirty-five control mice received 0.08 cc. of sesame oil per mouse weekly; a total of 1.7 cc. per mouse was administered from the second to the eighth month of age.

Experiment 5.—Thirty-five intact mice received a total of 20 mgm. of crystalline cholesterol per mouse.

Experiment 6.[1]—Thirty-five castrated mice received a total of 10 mgm. of crystalline progesterone per mouse.

Group III

Experiment 7.—Thirty-four control mice received 0.1 cc. of sesame oil per mouse weekly; a total of 1.6 cc. per mouse was administered from the second to the seventh month of age.

Experiment 8.[2]—Thirty-four intact mice received a total of 19 mgm. of cholestenone per mouse.

Experiment 9.[2]—Thirty-five castrated mice received a total of 19 mgm. of cholestenone per mouse.

In addition to the long-term experiments described above, the effect of estrone and progesterone alone and in combination upon the development of the mammary gland of virgin Marsh-Buffalo mice was studied.

Source and Purity of Steroids

The progesterone used in the first experiments (2 and 3) was especially made for us at our request, in the laboratories of Eli Lilly and Company, by the oxidation of cholesterol according to the method of Spielman and Meyer (12). No effort was made at further purification beyond that outlined in the method. The product contained about 60 per cent by weight of so-called inert material. In the preparation of progesterone by this method cholestenone is the main by-product, and has been tested in our present studies. Some androgenic material also is formed in a side reaction, and this is undoubtedly androstenedione; but the amount present in the sample used by us was negligible because of partial purification. One unit of this product given to castrated rats produced no effect upon seminal vesicles and prostate, and it required 10 units to double the weight of the former.

The cholesterol given in Experiment 5 was prepared from human gallstones by cold alcohol extraction, and had been recrystallized 3 times. The Lifschütz reaction, a very sensitive test for oxidative impurities, was negative. Spectrographic analyses of this cholesterol gave a different curve from the so-called C. P. preparations available on the market. Some of these have probably suffered denaturation.

The steroid in each instance was dispersed in sesame oil by addition of an ethanol solution of the steroid. The alcohol was evaporated by heating the oil at the temperature of the water bath.

[1] The crystalline progesterone administered in Experiment 6 was supplied by Parke, Davis and Company and by the Schering Corporation.

[2] The cholestenone used in Experiments 8 and 9 was supplied by the Abbott Laboratories and by the Schering Corporation (m.p. 79° to 81° C.).

Results of Long-Term Experiments

Group I

Tumors of mammary gland.—Progesterone had no influence upon mammary tumor formation in the intact mice (Experiment 2). While the cumulative incidence was 16 per cent lower than the control incidence at the 18th month of age the difference is not significant; fewer mice were available in the treated group for mammary tumor development because of the development of more lymphoid tumors and of malignant growths at the site of injection, and because of a higher death rate from causes not related to tumor formation. The 11 per cent incidence of mammary tumors in the castrated mice (Experiment 3) would appear to be significant, as Cori found no mammary tumors in Buffalo mice castrated at the same age (4). The uteri of the castrated mice that developed mammary tumors were rudimentary threads.

Lymphoid tumors.—The 21 per cent incidence of lymphoid tumors in the castrated mice (Experiment 3) would be significantly greater than the 3 per cent incidence found in the controls if other factors removing mice from the experiment had been balanced. However, since there were more than twice as many mice available for lymphoid tumor development in the castrated group as in the controls at the 16th month of age, the significance of the uncorrected accumulated incidences may be questioned.

Nonmalignant tumors.—A striking result of the experiment was the high incidence (60 per cent in Experiment 3, 34 per cent in Experiment 2) of plaque-like tumors at the site of injection of progesterone in sesame oil, and a complete absence of these tumors in the controls, which received only sesame oil. These tumors were ochre colored, oily in appearance, and rather friable; their average weight at autopsy was 400 mgm.; their range, from 100 to 1,600 mgm. On preparation of paraffin sections the greater portion disintegrated, indicating a high content of debris. The walls of these tumors were characterized by sheaths of fibrous tissue in which were incorporated vacuoles of various sizes; areas of myxomatous degeneration, of

necrosis and hemorrhage, foreign body giant cells, and nests and rings of fibroblasts were not uncommon. An analysis of these growths follows.

Analyses of oleomas.—At autopsy they were dissected free from the surrounding tissues, weighed, and preserved in 95 per cent ethanol. Approximately 7 gm. of this material was collected in each experiment, ground in a glass mortar, and extracted in the cold with 200 cc. of 95 per cent ethanol. The residue was taken up in 50 per cent ethanol and extracted twice with petroleum ether. An aliquot of all extracts was evaporated to dryness at room temperature and re-extracted, first with petroleum ether then with chloroform.

	Lipids, per cent	Cholesterol, per cent
Injected normal	8.5	0.33
Injected ovariectomized	10.2	0.41

Malignant tumors.—One control mouse developed a lymphosarcoma beneath an area of skin that may have been in contact with the sesame oil. In contrast 11 per cent of the mice in Experiment 2 and 21 per cent of those in Experiment 3 developed malignant tumors at the site of injection. In Experiment 3 there were 7 fibrosarcomas and 1 lymphosarcoma. One fibrosarcoma was a mixed tumor, with an area of adenocarcinoma. In Experiment 2 there were 3 fibrosarcomas and 2 plaques, the walls of which showed changes indicative of malignancy.

Group II

Tumors of the mammary gland.—Cholesterol given to intact mice had no influence upon the development of mammary tumors (Experiment 5). Crystalline progesterone given to mice ovariectomized at 23 days of age produced no tumors of the mammary gland.

Lymphoid tumors.—No effect upon lymphoid tumor formation was indicated.

Nonmalignant tumors.—In contrast to Experiment 2 and 3, plaques of inert material did not form at the site of injection in Experiments 5 and 6. There were, however, a number of deposits of oil, which were enclosed in a membrane. Sections of the membranes of 3 mice treated with cholesterol showed round cell infiltration, foreign body giant cells, slits, and spaces that had probably contained sesame oil. The analyses of the cholesterol content of the oil in these cysts follow; it should be noted, however, that the test for cholesterol, which was the conventional Lieberman reaction, is not specific for this compound but is given by the phytosterols also, and that the sesame oil contained an appreciable amount of the substance giving the test.

Oil cysts of	Age of mice, months	Total sterol, per cent	Sterol esters, per cent	Ratio
3 Control mice (Exper. 4)	15	2.53		
1 Control mouse (Exper. 4)	15	2.38	1.78	.75
1 Control mouse (Exper. 4)	15	2.6	1.36	.52
Blank for sesame oil		0.80		
1 Cholesterol-treated mouse (Exper. 5)	15	5.24		
1 Cholesterol-treated mouse (Exper. 5)	15	4.23	3.08	.73
1 Cholesterol-treated mouse (Exper. 5)	17	7.27	2.89	.40
1 Progesterone-treated mouse (Exper. 6)	17	2.80	1.59	.57
4 Progesterone-treated mice (Exper. 6)	17	3.95	2.29	.58

Malignant tumors.—There were no malignant tumors produced at the site of injection in Experiments 4, 5, or 6.

Group III

Tumors of the mammary gland.—In the intact mice (Experiment 8) that received cholestenone the cumulative incidence of mammary tumors was increased over that observed in controls (Experiment 7) by 20 per cent at the 12th and 13th months of age. The difference is twice the standard deviation of the mean, and would be considered significant. The final course of mammary tumor development was not different from that of the controls. The ovariectomized mice that received cholestenone did not develop mammary tumors.

Lymphoid tumors.—The increase in lymphoid tumor formation occurring at the end of the experiment in the ovariectomized mice is probably not significant, since none developed mammary tumors and more mice were available for lymphoid tumor formation.

Nonmalignant tumors.—Fourteen per cent of the controls (Experiment 7), 56 per cent of the treated intact mice (Experiment 8), and 47 per cent of the treated ovariectomized mice (Experiment 9) developed the ochre colored plaques at the site of injection. These have been described under Experiments 2 and 3 as consisting mainly of inert material.

Local malignant tumors.—One mouse in each of Experiments 7, 8, and 9 developed a fibrosarcoma at the site of injection.

TABLE I: CUMULATIVE INCIDENCE OF TUMOR FORMATION AND DEATH DUE TO OTHER CAUSES IN MARSH-BUFFALO MICE THAT RECEIVED A SERIES OF CHEMICALLY RELATED STEROIDS GIVEN AS PERCENTAGE OF CASES

	Exper. 1 Control (38 mice)				Exper. 2 Crude progesterone. Treated, intact (38 mice)				Exper. 3 Crude progesterone. Treated, ovariectomized (38 mice)			
Month	Ad. ca. mammary gland	Lympho-sarc.	Local cancer	Other causes	Ad. ca. breast	Lympho-sarc.	Local cancer	Other causes	Ad. ca. breast	Lympho-sarc.	Local cancer	Other causes
7				3								
8				3	3			6				3
9	8			3	6			6				3
10	8			3	13	6	6	6	3		8	3
11	11			6	13	6	6	6	6	3	11	3
12	18			6	18	8	8	6	6	3	13	3
13	34			6	34	11	11	6	8	6	18	3
14	42	3		6	42	11	14	13	8	8	18	3
15	60	3	3	6	48	11	14	13	11	11	18	3
16	63	3	3	6	48	11	14	18	11	13	18	3
17	63	3	3	6	48	11	14	21	11	18	21	3
18	66	3	3	6	50	11	14	21	11	21	21	3

	Exper. 4 Control (35 mice)				Exper. 5 Cholesterol. Treated, intact (35 mice)			Exper. 6 Crystalline progesterone. Treated, ovariectomized (35 mice)	
7							6		3
8	3				3	3	6		3
9	6			3	17	6	6		3
10	12	3		6	20	8	6		3
11	24	6		8	26	12	6		3
12	36	8		8	34	12	6		3
13	39	12		17	46	12	6		3
14	41	12		17	49	12	6	6	3
15	44	12		24	57	15	6	8	3
16	47	12		26	57	15	6	12	3
17	53	12		26	57	15	6	15	3
18									

	Exper. 7 Control (34 mice)				Exper. 8 Cholestenone. Treated, intact (34 mice)			Exper. 9 Cholestenone. Treated, ovariectomized (34 mice)	
7		3							
8		3			6			3	
9	3	3			9			3	
10	3	3			12			3	
11	6	3			23	3		3	3
12	12	3			32	3		3	3
13	15	3		3	38	3		3	9
14	38	3		3	44	6		12	9
15	47	3		6	56	9		17	9
16	56	3	3	9	59	9		26	9
17	62	3	3	9	61	9		26	9
18	62	12	3	9	68	12	3	32	12

Influence of estrone and progesterone on mammary gland development.—Twenty-four virgin female Marsh-Buffalo mice were segregated in 4 experimental groups at the age of 6 months. One group, receiving only injections of sesame oil, served as a control. One group received weekly injections of estrone in oil, another received progesterone in oil, and a third received a combination of these two hormones. Injections were made subcutaneously as in the long-term experiments. The period of treatment covered 5 weeks, and the total amount of hormone administered per mouse was 500 units of estrone and 3 (3 mgm.) of progesterone. Whole mounts of the lower mammary gland of each mouse were prepared and a 0, 1, 2, 3, 4 classification was made on the basis of 3 objective measures: number of ducts, width

of ducts, and number of alveoli. The results are given in Table II. The results show that: (a) the

TABLE II: INFLUENCE OF ESTRONE, PROGESTERONE, AND THEIR COMBINATION UPON MAMMARY GLAND DEVELOPMENT IN THE SIX MONTH VIRGIN MARSH-BUFFALO MOUSE

Treatment	Histology of Mammary Gland 0, 1, 2, 3, 4 Classification		
	No. of ducts	Width of ducts	No. of alveoli
Control	2.7 ± 0.5	2.4 ± 0.5	2.0 ± 0.6
Estrone, 500 u. per mouse	2.7 ± 0.3	1.9 ± 0.3	2.2 ± 0.5
Progesterone, 3 mgm. per mouse	2.5 ± 0.3	2.2 ± 0.4	2.7 ± 0.6
Estrone and progesterone as above	3.2 ± 0.3	1.7 ± 0.3	2.5 ± 0.4

7 months old female of the Marsh-Buffalo strain may show no alveolar development excepting terminal buds (confirming previous work); (b) neither estrone, progesterone, nor a combination of the two produced any demonstrable effect upon ductal or alveolar development.

DISCUSSION

Progesterone and carcinogenesis.—Heiman (8) has shown that in the RIII strain progesterone in the dose range used in our experiments has a pronounced inhibitory effect upon the incidence of mammary tumors; none appeared in castrated females of this strain that had received the hormone. Our results, therefore, agree with his, in that progesterone is noncarcinogenic, but the inhibitory effect shown for strain RIII is obviously not encountered with Marsh-Buffalo mice, which have previously shown (2, 3) a notable resistance to the carcinogenic effect of estrogens. This resistance is substantiated by their failure to respond to estrone and progesterone both alone and in combination with development of the mammary glands, as recorded in the present experiments. Heiman's interpretation of his results is that the hormones employed probably reduced the pituitary gonadotropic fraction, and that this deficiency was in turn followed by a suppression of ovarian secretion. In this respect it is interesting to note that we were able to suppress ovarian secretion, and thus reduce the incidence of cancer, by long-continued administration of mare serum and sheep pituitary gonadotropins, but not by the administration of human chorionic gonadotropin (2).

Sterol exchange from the sesame oil depot.—The analyses of oil cysts from mice that had received sesame oil without steroid showed a total cholesterol content, estimated by the Lieberman reaction, of 2.4 to 2.6 per cent. The blank for the sesame oil was 0.8 per cent, so that the accumulation of cholesterol-like material from the body fluids is indicated. The high ester content is characteristic of blood. In the mice that received cholesterol in a concentration of 1.2 per cent, the cholesterol content of the oil cysts varied from 4.2 to 7.3 per cent, indicating that the presence of cholesterol increased the deposition of extra cyst cholesterol above that which formed in the cholesterol-free oil. The influence of crystalline progesterone is intermediate. These analyses show rather conclusively that in the oil cyst there can be both a release and an accumulation of steroid; in other words, the cyst is in equilibrium with the steroid system of the body fluids. These analyses should be contrasted with those of the oleomas that formed after the injection of sesame oil containing impure progesterone or cholestenone. In these the cholesterol content was only 0.3 to 0.4 per cent and the total lipid content 8.5 to 10.2 per cent. The ratio of cholesterol to total lipid is of the same order, so that in the oleoma water, protein, and electrolytes have to a large extent replaced fat. One would be tempted to ascribe the formation of the nonmalignant plaque to the presence of cholestenone, since the plaques formed to considerable extent only in those experiments in which pure cholestenone was administered or was present as an impurity. There is no evidence that cholestenone contributed to the formation of malignant tumors at the site of injection. Kirby (9), in his experiments on feeding derivatives of heated cholesterol, states that cholestenone is noncarcinogenic. However, it should be noted that in Experiment 8, in which intact mice received pure cholestenone, the onset of mammary tumors was hastened, statistical analyses of the data indicating that the observation was significant.

Local tumors.—The production of cysts following the intramuscular injection of vegetable oils is well known (10). It has been shown (6) that estrone is resorbed from a sesame oil depot in from 3 to 9 days. Though it is common practice in many research laboratories to aspirate the oil cysts that form in the course of an experiment we have not done so; it is apparent that the formation of a cyst from a subcutaneous injection gives opportunity for the local development of the carcinogenic process depending on the steroid content, and thus offers a valuable tool in the study of cancer. In the large series of experiments in which we have injected estrogens in sesame oil, the formation of local skin tumors (usually fibrosarcoma) was never above the normal incidence in females. In comparing Experiments 3, 6, and 9, which are concerned with ovariectomized mice, it is revealed that in Experiment 3, 21 per cent of the mice developed malignant tumors at the injection site (which figure does not include the 11 per cent that developed adenocarcinomas), while no malignant

tumors whatsoever appeared here in Experiments 6 and 9. The mice of Experiments 6 and 9 received crystalline progesterone or crystalline cholestenone, and *the crude progesterone of Experiment 3 is thus proved to contain a carcinogenic element,* since 21 per cent is 3 times the standard deviation of the mean. If the 11 per cent incidence of adenocarcinoma is added, the ratio of 32 to 0 per cent becomes formidable. There is every reason to believe that the adenocarcinoma was a local effect, since histological study indicated that the mammary gland areas came in contact with the developing oil plaques, and the uteri remained infantile, showing the absence of estrogens. It should be stressed that there is a tendency in female Marsh-Buffalo mice to form tumors of the skin (usually fibrosarcoma), and the appearance of 1 fibrosarcoma each in Experiments 1, 2, and 7, and none in Experiments 4, 5, and 6, giving an incidence of 3 out of 245 mice, or 1.2 per cent, is normal. In previous experiments 2 fibrosarcomas developed in 176 female mice (1.1 per cent) injected with estradiol in sesame oil. Of 95 castrated males, 2 developed a fibrosarcoma (2.2 per cent). These data are compiled to the age of 17 to 18 months, when the experiments were terminated. In previous experiments male mice observed for 20 months or more developed a higher incidence of skin tumors; namely, 10 per cent in 30 control mice, and 23 per cent in 30 males that had received estrone. Steiner and his group (13) found that 61 mice of heterogeneous stock injected with sesame oil as a vehicle for other substances failed to develop skin tumors. On the other hand, 3 of 9 mice developed sarcoma when injected with sesame oil heated to 350° C.

Endogenous production of carcinogens.—The product that exerted a local carcinogenic effect in our experiments was the crude progesterone made by the method of Spielman and Meyer from cholesterol by oxidation of the dibromide of cholesterol in benzene solution with aqueous permanganate. The relation of the reaction products is given in Fig. 1. Cholesterol, cholestenone, and progesterone have been eliminated as carcinogenic agents by the results of the present experiments. Androstenedione is eliminated by its absence in any amount exceeding a trace in the material used. Suspicion rests, therefore, on the large series of possible intermediate compounds that theoretically could be formed in either of the paths of degradation. The progress of the research is reported to this point: Oxidation and reduction of cholesterol dibromide according to the procedure of Spielman and Meyer gave a product that was carcinogenic. It is considered worth while to pursue the problem farther, since the reactions in the Spielman-Meyer procedure are not unlike those that conceivably could occur in the organism. The accidental discovery that this process leads to a carcinogen is considered a valuable clue in the effort to banish the spectre that exists at present: The question whether or not carcinogens are endogenously produced from cholesterol.

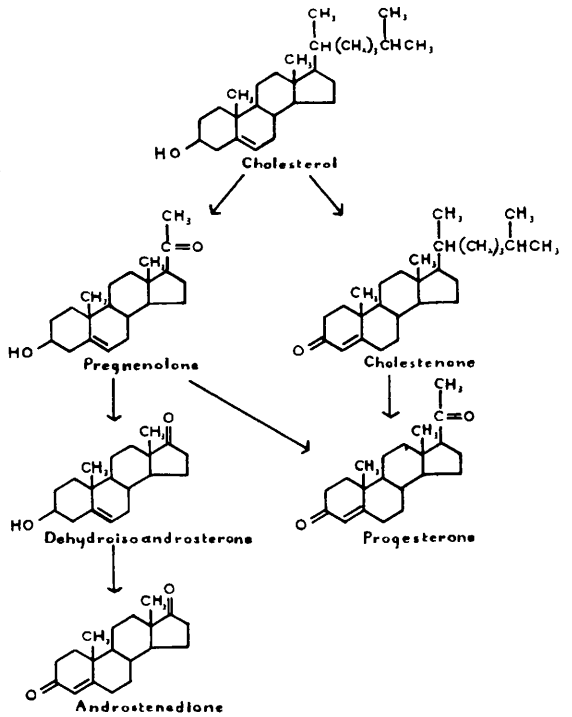

FIG. 1.—Intermediary derivatives and side reaction derivatives in the degradative oxidation of cholesterol to progesterone. In the chemical procedure the dibromide of cholesterol is used and bromine is later removed with zinc. This should be borne in mind, as it opens the possibility of reduction as well as oxidation.

SUMMARY

1. Progesterone, under the conditions that inhibited mammary cancer formation in RIII mice, failed to do so in Marsh-Buffalo mice.

2. Ovariectomized Marsh-Buffalo mice that received 10 mgm. of progesterone per mouse subcutaneously over a period of 6 months failed to develop mammary tumors.

3. Three milligrams of progesterone administered subcutaneously, alone or in combination with 500 units of estrone, failed to effect development of the mammary glands in Marsh-Buffalo female mice.

4. Mice that received sesame oil containing crude progesterone contaminated with cholestenone, or containing the equivalent of crystalline cholestenone, developed oleomas at the site of injection. Crystalline cholesterol or progesterone under the same conditions was without influence.

5. A crude synthetic progesterone made by the method of Spielman and Meyer resulted in a 32 per cent incidence of malignant tumors at the site of injection, compared with a 0 per cent incidence in controls and a 1 to 2 per cent incidence in the colony.

REFERENCES

1. Allen, E. Estrogenic Hormones in the Genesis of Tumors and Cancers. Endocrinology, **30**:942-952. 1942.
2. Bischoff, F., Long, M. Louisa, Rupp, J. J., and Clarke, G. J. Endocrine Factors Influencing Tumor Development. The Effect of the Gonadotropins and of Theelin upon the Marsh-Buffalo Adenocarcinoma and Lymphosarcoma. Endocrinology, **28**:769-779. 1941.
3. Bischoff, F., Ingraham, Louise P., and Clarke, Georgena J. Influence of Nontoxic Amounts of Estradiol upon Carcinogenesis in Intact and Castrated Male and Female Mice of the Marsh-Buffalo Strain. Cancer Research, **5**: 579-581. 1945.
4. Cori, C. F. The Influence of Ovariectomy on the Spontaneous Occurrence of Mammary Carcinomas in Mice. J. Exper. Med., **45**:983-991. 1927.
5. des Ligneris, M. A. J. The Production of Benign and Malignant Skin Tumours in Mice Painted with Bantu Liver Extracts. Am. J. Cancer, **39**:489-495. 1940.
6. Emery, F. E., Mathews, C. S., and Schwabe, E. L. The Absorption of Stilbestrol and Theelin from Cysts of Sesame and Peanut Oils. J. Lab. & Clin. Med., **27**:622-627. 1942.
7. Fieser, L. F. The Chemistry of Natural Products Related to Phenanthrene. New York: Reinhold Publishing Corporation. 1936, pp. 86, 251-256.
8. Heiman, J. The Effect of Progesterone and Testosterone Propionate on the Incidence of Mammary Cancer in Mice. Cancer Research, **5**:426-430. 1945.
9. Kirby, A. H. M. Attempts to Induce Stomach Tumors. I. The Effect of Cholesterol Heated to 300° C. Cancer Research, **3**:519-525. 1943.
10. Page, R. C., and de Beer, E. J. Production of Cysts Following Intramuscular Injection of Vegetable Oils. Am. J. M. Sc., **205**:812-814. 1943.
11. Roffo, A. H. Pirólisis de colesterol; alquitrán cancerígeno del colesterol. Bol. Inst. de med. exper. para el estud. y trat. d. cancer, **18**:929-943. 1941.
12. Spielman, M. A., and Meyer, R. K. The Preparation of Progesterone from Cholesterol. J. Am. Chem. Soc., **61**: 893-895. 1939.
13. Steiner, P. E., Steele, R., and Koch, F. C. The Possible Carcinogenicity of Overcooked Meats, Heated Cholesterol, Acrolein, and Heated Sesame Oil. Cancer Research, **3**: 100-107. 1943.