THE EFFECT OF COLCHICINE AND X-RAY ON A TRANSPLANTABLE MAMMARY CARCINOMA IN MICE*

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The first observations that the alkaloid colchicine affected the growth of neoplastic tissue were made by Dominici.[7] He stated that patients with gout and cancer who were receiving colchicine showed a remarkable improvement in health. Amoroso[1] also noted similar beneficial effects in gouty patients with cancer. He reported complete retrogression of grafted tumors in mice which had received repeated small doses of colchicine subcutaneously.

Dixon and Malden[6] observed marked leukocytosis in rabbits after administration of colchicine, and Dixon[5] in his *Manual of Pharmacology* stated that colchicine was a substance that excited mitosis. Lits[12] and Dustin[8, 9] studied its effect on transplantable mouse tumors and observed a marked increase in mitotic figures, followed subsequently by pyknosis and necrosis of many of the dividing cells. They concluded that colchicine has a double action; first, it explosively stimulates mitotic division in all cells having the potentialities of division and, secondly, it arrests these dividing cells at the metaphase for a period of several hours and in large doses actually destroys them. They consider its action similar to that of several other substances previously studied by them which they call "caryoclastic poisons."

Ludford[14] concluded that colchicine prevented the formation and proper function of the mitotic spindle. Thus, cells starting to divide while under the influence of colchicine are unable to complete their division and are arrested at the metaphase. The result is an accumulation of cells at the metaphase during the period of blockade, and this accounts for the apparent increase in mitotic activity. The studies of Brues[2] on the effect of colchicine on regenerating liver in rats tend to support the observations of Ludford.[14]

Oughterson, Tennant, and Hirshfeld[15] observed a typical colchicine effect with a marked increase in the number of mitoses in a variety of human tumors after subcutaneous injection of from 2 to 5 mg. of the alkaloid.

Attempts to produce regression of transplantable and spontane-

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ous tumors in mice and in other animals with either single or repeated doses of colchicine have been reported by several workers. Peyron et al.[17, 18, 19] used repeated doses of colchicine and obtained regression of the Shope rabbit papilloma. Ludford[14] and Clearkin,[4] using mouse tumors, failed to produce regression with the doses employed, and Ludford stated that he was unable to affect the growth of the tumor except with doses that were toxic for the animals. Poulsson[21] treated ten tar cancers and five spontaneous mammary cancers in mice with a dose of 1/80 mg. injected daily for 17 days without any effect on either the tumors or the mice. Lits, Kirschbaum, and Strong[13] reported regression of subcutaneous transplants of mouse leukemia with repeated doses of colchicine, but the tumors subsequently recurred in all instances and killed the animals. Nevertheless, a definite prolongation of life was obtained in the treated animals.

It has been well established that dividing cells are more sensitive than are resting ones to the injurious effects of x-radiation. Colchicine affords a method of holding a large number of the cells in a tumor at the metaphase for several hours. It seemed worth while, therefore, to combine colchicine and x-ray in the treatment of tumors with the hope of finding that a combination of the two agents would be more effective than x-ray alone.

The response of Yale carcinoma No. 1 to x-ray has been thoroughly studied by Oughterson, Tennant, and Lawrence.[16] The present paper deals with the effect of carefully controlled doses of colchicine on the tumor and also the effects of a combination of colchicine and x-radiation.

Materials and methods

Unless otherwise specified, young adult albino mice of the A strain, varying in weight from 14 to 20 gm., were used. The animals were weaned at approximately 8 weeks of age and were then maintained upon a diet of Nurishmix. The donor tumor, Yale carcinoma No. 1,[10] was removed sterilely, cut in pieces 1 to 2 mm. in diameter, and then inoculated subcutaneously in the back or abdomen of the experimental animals. When the tumor had attained a diameter of 0.8 to 1 cm., usually in 10 to 14 days, the animals were weighed and injected with colchicine. In the x-radiation experiments the mice were used when the tumor reached a size of 0.5 to 0.7 cm.

Colchicine alkaloid (Mallinckrodt) was kept in the dark in a refrigerator. Shortly before use small amounts were accurately weighed and dissolved in physiological saline solution. The colchicine dose was calculated in mg. per gm. of mouse and was given subcutaneously in a total volume of 0.5 cc. of saline. The animals were killed at definite intervals following injection; the

tumors were removed immediately and fixed in either formalin or Zenker acetic solution.

Experiments

The experiments may be divided into four groups as follows:

I. Experiments on dosage.
   A. Effect of a single dose of varying size at varying intervals.
   B. Effect of doses of varying size repeated at 9-hour intervals.
II. Attempts to cause regression of the tumors with either single or repeated doses.
III. The effects of colchicine on spontaneous tumors of A-strain mice.
IV. The effect of colchicine plus x-radiation.

Results

I. EXPERIMENTS ON DOSAGE

A. *Effect of a single dose of varying size at varying intervals.*

In these experiments groups of approximately 10 mice received respectively doses of 0.0007, 0.0008, 0.0009, 0.001, 0.0012, 0.0014, 0.0016, and 0.0035 mg. of colchicine per gm. of body weight and were killed 9½ hours after injection. This series of experiments yielded three facts:

1. The tumors in mice receiving 0.0035 mg. of colchicine per gm. were swollen, blue, hemorrhagic, and soft; the animals appeared ill. Microscopically the center of the tumor was hemorrhagic and necrotic. A thin border of viable tumor cells surrounded the central necrotic zone. Some of these cells were undergoing division. They were all in the metaphase, with the chromosomes dark and closely packed together (Fig. 1).

2. In those mice receiving 0.0007 mg. or less of colchicine per gm. the tumors were indistinguishable from those in the controls, while tumors whose hosts had received 0.0008 mg. of colchicine per gm. exhibited only a slight colchicine effect.

3. With doses between 0.0009 and 0.0012 mg. per gm. consistent colchicine effects were uniformly obtained. These effects were as follows: (a) No change in the gross appearance of the tumor. (b) Microscopically, from one-half to three-quarters of the tumor cells were undergoing division. The majority of the dividing cells were arrested at the metaphase. Only an occasional telophase was seen. (c) The chromosomes were closely packed, giving a contracted appearance to the mitotic figures. (d) There were occasional abnormal mitotic figures with irregular dispersion of the chromosomes throughout the cytoplasm and the formation of micronuclei. (e) Necrosis was absent.

A second group of experiments was designed to determine the effect of colchicine at varying intervals following its injection. Groups of mice received doses of 0.0008, 0.0009, 0.001, 0.0012, 0.0014, and 0.0035 mg. per gm. and were killed at intervals of 1, 2, 3, 4, 5, 9, 12, and in some cases 17, 21, 25, 48, and 63 hours. Four mice in each dosage group were killed for study at each time interval.

The results may be divided into three groups:

1. In those mice receiving a dose of 0.0035 mg. per gm. the tumors exhibited as early as 1 hour after injection a definite colchicine effect in the form of contracted metaphases. The number of cells at the metaphase gradually increased up to 5 hours when it was estimated as a ++ effect. At this time central necrosis appeared and while this increased, reaching a maximum at 9 hours, the number of metaphases never exceeded ++. The tumors at 9 hours were similar to those described in the preceding section, i.e., they showed a marked central necrosis with a peripheral area of viable tumor cells, a few of which were in division and were at the metaphase. The contracted metaphase had disappeared by 12 and 17 hours, leaving a border of viable tumor cells surrounding a central necrotic mass. The findings were similar in sections taken at 21, 25, 48, and 63 hours.

2. In the mice receiving doses of 0.0009 to 0.0014 mg. per gm. the necrosis decreased as the dose of colchicine was decreased. With a dose of 0.0014 mg. per gm. it was only one-plus (+) and did not manifest itself until 9 hours after injection. In doses smaller than this necrosis was not observed. With all of these doses a definite colchicine effect was noted early in the form of arrested and contracted metaphases. With a dose of 0.0014 mg. per gm. this never exceeded ++ but the changes persisted to some extent until 21 hours. The most striking results were found with doses of 0.0012, 0.001, and 0.0009 mg. per gm. The effect of these doses manifested itself in the form of marked mitotic arrest at the metaphase, becoming noticeable at 1 or 2 hours, reaching a maximum around 6 or 9 hours, and decreasing after 12 or 13 hours (Fig. 2).

3. The mice receiving a dose of 0.0008 mg. per gm. differed from group 1 in that necrosis was completely absent. A colchicine effect manifested itself early in the form of contracted metaphases and was graded as + at 2 hours, ++ at 3 hours and +++ at 4, 5, and 6 hours. The effect of the drug had begun to pass off by 9 hours, as indicated in the marked decrease in the number of metaphases.

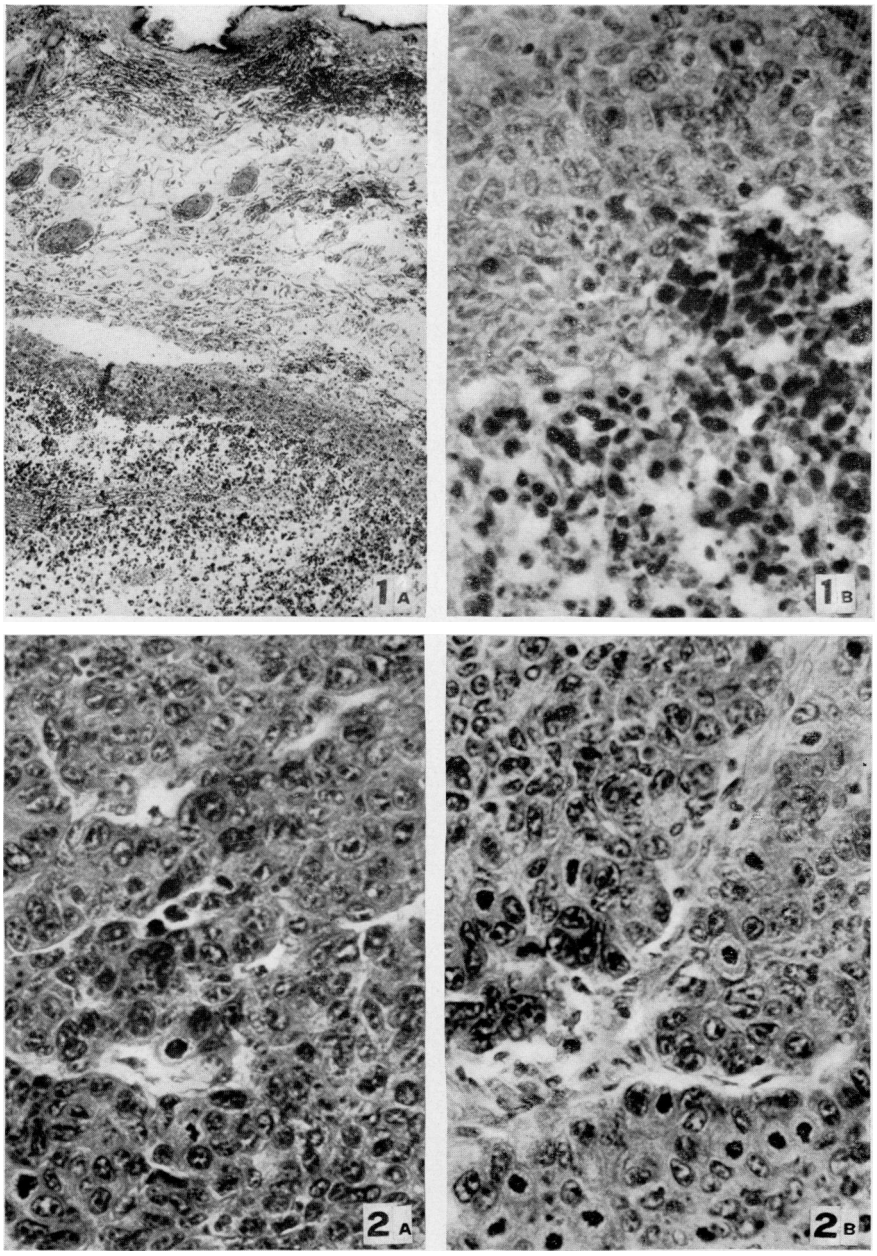

FIG. 1. (A). The effect of a dose of 0.0035 mg. of colchicine per gram of body weight on Yale carcinoma No. 1, 9½ hours after injection. The center of the tumor is necrotic. A thin border of viable tumor cells surrounds the central necrotic zone. The dividing cells are all in the metaphase.

(B). High power view of *a* showing junction of necrotic center with border of viable cells.

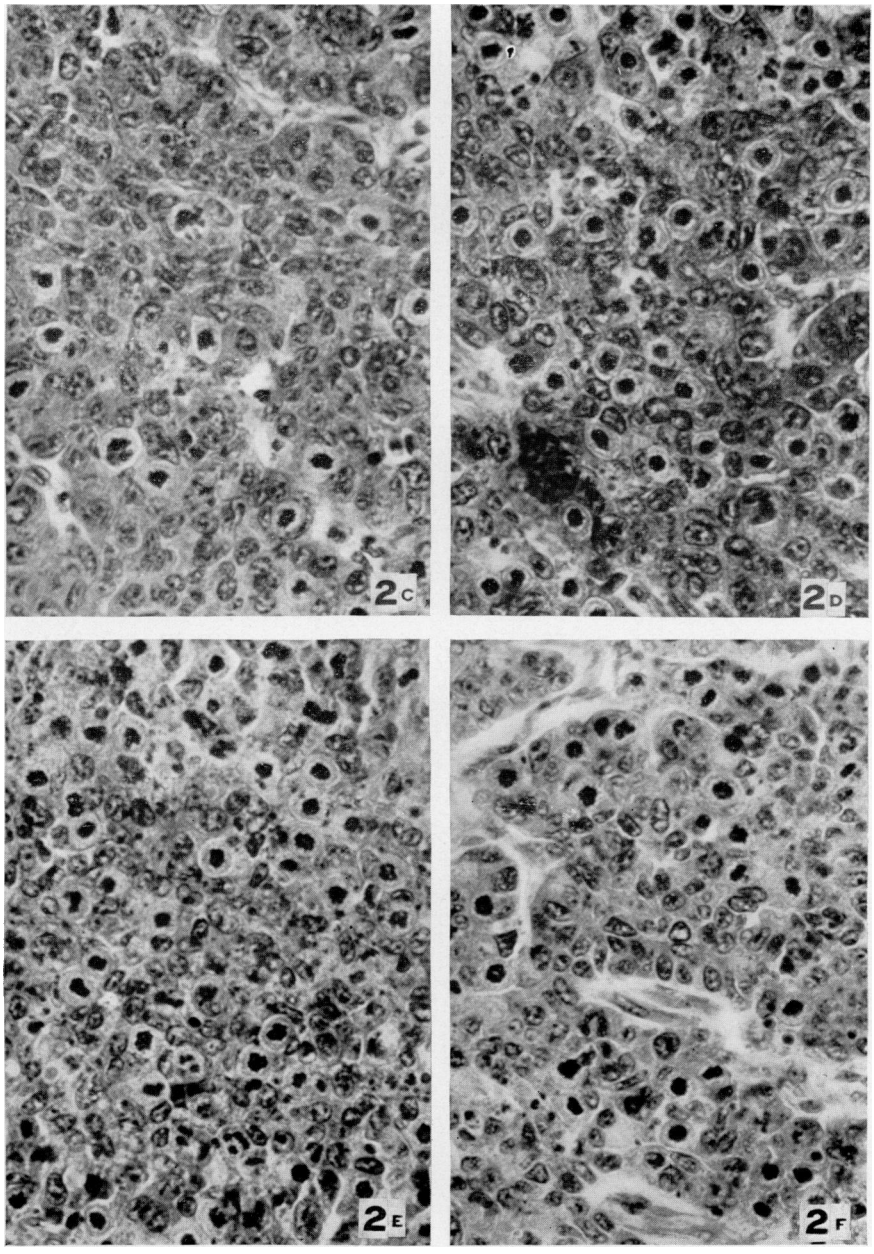

FIG. 2. The effect of a dose of 0.0012 mg. of colchicine per gram of body weight on Yale carcinoma No. 1 after varying intervals of time. (H and E) × 100.
A. Control B. 1 hour C. 2 hours D. 5 hours E. 9 hours F. 12 hours

B. *Effect of doses of varying size repeated at 9-hour intervals.*

In an attempt to increase the number of cells at the metaphase the influence of repeating the dose at the height of the reaction (9-hour interval) was tried. Doses of varying size were injected several times at 9-hour intervals. Two mice were sacrificed at the end of each 9-hour period in each dosage group. An injection of 0.0007 mg. per gm. was repeated 9 times at 9-hour intervals before the mice succumbed to the toxic effects of the drug. Marked increase in mitoses was obtained at 27 and 36 hours, i.e., 9 hours after the second and third doses. All of the cells were not in division, however, and necrosis appeared at the 36th hour. By the 45th hour (9 hours after the fourth dose) this was marked and increased steadily until the 81st hour when the animals died. The same general effect was noted with doses of 0.0009 and 0.001 mg. per gm., although the maximum increase in mitoses and necrosis appeared earlier. With doses as large as 0.0014 mg. per gm. marked necrosis was apparent by the 18th hour, or 9 hours after the second dose, and all of the animals died after the sixth dose.

II. ATTEMPTS TO CAUSE REGRESSION OF THE TUMORS WITH SINGLE OR REPEATED DOSES.

Fifteen mice were used for an experiment, 10 receiving the drug and 5 being retained as controls for each of the doses. The doses are given in Table 1.

TABLE 1

Dose of colchicine mg./gm.	*Interval between doses*	*Number of doses*
0.0035	.....	1
0.0020	4 days	7
0.0016	3 "	3
0.0016	3 "	4
0.0016	3 "	8
0.0016	4 "	4
0.0016	4 "	8
0.0016	4 "	9
0.0016	5 "	10
0.0016	5 "	13
0.0010	9½ hrs.	4X
0.0010	Given once, repeated in 9½ hours, then after an interval of 5 days a second course was given; 7 courses were administered.	

In no instance did regression of the tumor occur and with most of these doses the animals succumbed to the toxic effects of the drug. With a dose of 0.0016 mg. per gm. repeated at intervals of 3 to 5 days some retardation in the growth of the tumor and a prolongation of life were observed. In all cases, however, the dose necessary to accomplish this approached closely a lethal dose, since a significant number of the mice succumbed to its toxic effects. In a few instances the solution was injected directly into the tumor. Some of the tumors decreased in size, but they subsequently grew and killed the mice.

III. The effect of colchicine upon spontaneous tumors of A-strain mice.

Twenty-six spontaneous tumors occurred in the mouse colony during the course of the work. These mice were given 0.0010 mg. of colchicine per gm. and then killed after an interval of 9½ hours. All of the tumors showed a typical colchicine response comparable to that found in the transplanted tumors. The more malignant, rapidly growing tumors had many cells in metaphase while the slower-growing, well-differentiated tumors exhibited a much smaller number of mitotic figures.

Some of the spontaneous tumors were treated with repeated small doses of colchicine, either 0.001 or 0.0016 mg. per gm., injected into the tumor. No regressions were obtained.

IV. The effect of colchicine plus x-radiation.

Groups of mice with tumors, 0.4 to 0.5 cm. in diameter, on the dorsum of the thigh were irradiated 8 to 10 hours after receiving a single dose of 0.0016 or 0.0010 mg. per gm. of colchicine subcutaneously. Mice were also irradiated 8 to 10 hours after receiving the second of two doses of 0.0010 mg. per gm. of colchicine which had been given at an interval of 9½ hours. The mice were anesthetized with seconal and irradiated in pairs. Two doses of x-ray were used, 2500 R and 5000 R. (The radiation factors were 90 KV, 4 m.a., 23.5 cm. distance, no filtration, size of port 2 cm. cone, R output 149 R per minute.)

The results are given in Table 2.

TABLE 2

RADIATION OF TUMORS WITH A SINGLE DOSE OF 2500 R

Dose of colchicine mg./gm.	*No. of mice treated*	*No. of mice dead from tumor*	*No. of mice cured*	*% of mice cured*
0.0016	19	16	3	10
0.0010	9	9	0	0
0.0010 2X	44	41	3	7
Radiation alone[16]	83	82	1	1
RADIATION OF TUMORS WITH A SINGLE DOSE OF 5000 R				
0.0016	8	4	4	50
0.0010	9	7	2	22
0.0010 2X	10	4	6	60
Radiation alone[16]	81			48

A higher percentage of cures was obtained with colchicine plus a dose of 2500 R than with x-radiation alone.[16] With colchicine plus a dose of 5000 R the results were similar to those with x-ray alone. Under these conditions colchicine does not strikingly increase the destructive effect of x-ray upon this tumor.

Summary

These experiments demonstrate that different colchicine effects may be obtained in the same tumor, depending upon the size of the dose. In order to obtain comparable and consistent histological changes it is necessary to calculate doses by weight. The failure to do this may account for much of the confusion which exists concerning the action of colchicine on tumors.

In experiments employing 0.0009, 0.0010, or 0.0012 mg. per gm., observation at hourly intervals showed that there was a progressive increase in the number of contracted metaphases starting one hour after injection and reaching a maximum in 9½ hours. This was followed by a gradual decline. These facts support Ludford and Brues' contention that the increase in mitoses is essentially a blockade at the metaphase with accumulation.

Large doses of colchicine (0.0020 mg. per gm.) produced extensive central necrosis and hemorrhage in Yale carcinoma No. 1. A border of viable tumor tissue always persisted. Smaller doses of colchicine (0.0016) repeated at 3- to 5-day intervals resulted in a moderate prolongation of life, but in all cases the animals eventually succumbed to the tumors.

The effect of combining x-ray and colchicine has been reported by Brues, Marble, and Jackson[3] and by Guyer and Claus.[11] Brues stated that colchicine is ineffective in enhancing the action of x-ray on tumors, while Guyer, using doses apparently lethal, found the combination to be more effective. In the present experiments, a dose of colchicine which produced a maximum accumulation of mitoses plus a single dose of x-ray of 2500 R gave a slightly higher rate of curability than did x-ray alone. However, it was not considered sufficiently significant to warrant further investigation.

References

1 Amoroso, E. C.: Colchicine and tumor growth. Nature, 1935, *135*, 266-67.

2 Brues, A. M.: The effect of colchicine on regenerating liver. J. Physiol., 1936, *86*, 63*P*-64*P*.

3 Brues, A. M., Marble, B. B., and Jackson, E. B.: Effects of colchicine and radiation on growth of normal tissues and tumors. Am. J. Cancer, 1940, *38*, 159-68.

4 Clearkin, P. A.: Effect of colchicine on normal and neoplastic tissues in mice. J. Path. & Bact., 1937, *44*, 469-80.

5 Dixon, W. E.: *A Manual of Pharmacology*. E. Arnold, London, 1906, p. 95.

6 Dixon, W. E., and Malden, W.: Colchicine with special reference to its mode of action and effect on bone-marrow. J. Physiol., 1908, *37*, 50-76.

7 Dominici, A.: [In] *Thérapeutique médicale*: *V*. Peau, Syphilis, Cancer. By M. Loeper, G. Milian, et al. Masson & Cie., Paris, 1932.

8 Dustin A. P.: L'action de la colchicine sur les tumeurs malignes. Leeuwenhoek Vereeniging, 4e conf., Amsterdam, 1935.

9 Dustin, A. P.: Contribution à l'étude de l'action des poisons caryoclasiques sur les tumeurs animales. Action de la colchicine sur le sarcome greffé, type Crocker, de la souris. Bull. Acad. roy. de méd. de Belgique, 1934, *14*, 487-502.

10 Gardner, W. U., Smith, G. M., Allen, E., and Strong, L. C.: Cancer of mammary glands induced in male mice receiving estrogenic hormone. Arch. Path., 1936, *21*, 265-72.

11 Guyer, M. F., and Claus, P. E.: Irradiation of cancer following injection of colchicine. Proc. Soc. Exper. Biol. & Med., 1939, *42*, 565-68.

12 Lits, F. J.: Recherches sur les réactions et lésions cellulaires provoquées par la colchicine. Arch. internat. de méd. expér., 1936, *11*, 811-901.

13 Lits, F. J., Kirschbaum, A., and Strong, L. C.: Action of colchicine on a transplanted malignant lymphoid neoplasm in mice of the C_3H strain. Am. J. Cancer, 1938, *34*, 196-213.

14 Ludford, R. J.: Action of toxic substances upon the division of normal and malignant cells in vitro and in vivo. Arch. f. exper. Zellforsch., 1936, *18*, 411-41.

15 Oughterson A. W., Tennant, R. Jr., and Hirshfeld, J. W.: Effect of colchicine on human tumors. Proc. Soc. Exper. Biol. & Med., 1937, *36*, 661-64.

16 Oughterson, A. W., Tennant, R. Jr., and Lawrence, E. A.: The tumor response and stroma reaction following x-ray of a transplantable tumor in inbred strains of mice. Yale J. Biol. & Med., 1940, *12*, 419-25.

17 Peyron, A., Lafay, B., and Poumeau-Delille, G.: Sur la régression du papillo-épithéliome du lapin (tumeur de Shope) sous l'action de la colchicine. Compt. rend. Acad. d. Sci., 1937, *205*, 378-80.

18 Peyron, A., Poumeau-Delille, G., and Lafay, B.: La tumeur de Shope du lapin et sa stérilisation par la colchicine. Bull. Assoc. franç. p. l'étude du cancer, 1937, *26*, 625-34.

19 Peyron, A., Poumeau-Delille, G., and Lafay, B.: Sur l'évolution maligne du papillo-épithéliome du lapin et son mode de régression sous l'action de la colchicine. Compt. rend. Soc. de biol., 1937, *126*, 685-88.

20 Poulsson, K. T.: Colchicine in treatment of malignant tumors in mice. Norsk. mag. f. lægevidensk., 1935, *96*, 735-36.