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Irradiation of Cancer Following Injection of Colchicine.*

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Inasmuch as cancer cells are reputed to be most vulnerable to the effects of X-rays during prophase and metaphase stages of division, and since the drug colchicine arrests mitosis in metaphase and holds it there for from 15 to 25 hours, it seemed probable that a colchicine treatment of animals bearing cancers followed some hours later by irradiation of the tumors should be a more effective treatment than X-ray treatment alone. With this thought in mind, beginning in July, 1938, the authors have experimented on 672 cancer-bearing rats including controls.

It speedily became evident that a dosage of colchicine of 0.2 mg or more per 100 g of body weight was likely to be harmful or even lethal for rats, particularly for females. A dosage of 0.1 mg per 100 g of body weight administered by subcutaneous injection, was settled upon as the standard in most of our experiments. Also, it was found that 3000 *r* of X-rays given at a single treatment were invariably lethal in from 2 to 3 weeks. While even 4500 *r* were tolerated when administered in single treatments of 1500 *r* each, if intervals of 2 weeks or more were allowed between exposures, as the experiments proceeded it became evident that smaller dosages (188 to 375 *r*) frequently repeated were more effective than larger and fewer dosages.

To determine the effects of various amounts of the drug, and particularly, the number of hours after administration that the maximal effect occurs, biopsies were made from the tumors of 5 rats at intervals of 5 hours and the number of mitotic figures in 3000 cells were counted each time in comparable fields of the same tumor. In the animal injected subcutaneously with 0.1 mg of colchicine per 100 g of body weight for example, the carcinoma showed before treatment an average of 6.3% of mitotic figures in a count of 3000 cells if one considered every cell to be in mitosis that revealed definite chromosomes. The count had risen to 15.9% at the end of 5 hours; to 20.2% at the end of 10 hours; and to 38.2% at the end of 15 hours. This was the maximum attained in this tumor. By the end of 45

* This investigation was aided by support from the Jonathan Bowman Memorial Fund, the Wisconsin Research Foundation, the Brittingham Trust Fund, and a Research Grant from Elizabeth Hopkins Johnson.

TABLE I.
Relative Percentage of Mitosis Seen at 5-hour Intervals After Administration of Colchicine.

No. of cells counted	Hr after treatment	Percentage of mitosis following administration of amts of colchicine specified per 100 g body-wt				
		.2 mg	.15 mg	.1 mg	.05 mg	.01 mg
3000	(control) 0	5.4	6.8	6.3	9.2	6.7
3000	5	9.2	—	15.9	18.0	14.1
3000	10	20.4	21.7	20.2	22.3	13.5
3000	15	24.0	40.3	38.2	—	22.7
3000	20	51.3	31.1	19.1	41.5	17.7
3000	25	64.8	—	19.8	17.6	—
3000	30	47.8	25.6	15.6	12.3	13.8
3000	35	32.2	18.0	18.4	14.5	14.8
3000	40	—	16.0	12.3	—	11.2
3000	45	—	15.2	11.0	16.8	10.1
3000	50	8.2	15.3	—	16.9	9.0

hours the count had returned to an average of 11.0%. In biopsies of tumors from other rats (not recorded in Table I) the influence of the drug as shown by noticeably increased numbers of mitotic figures persisted in some cases beyond 72 hours.

The greater lethal effect of combined colchicine and X-ray treatment over X-ray treatment alone was most strikingly demonstrated in experiments in which cancerous tissues from rats previously given 0.2 mg of colchicine per 100 g of body weight, along with comparable carcinomatous tissues from untreated animals, were submitted to exactly the same irradiation before implantation. In our experiments both sets of tissue were removed at the same time, cut into equally thin slices and given an exposure of 1500 *r* simultaneously in the same dish. The results are set forth in Table II. They show clearly the far greater lethal effect of the combined colchicine and X-ray treatment on carcinoma than that of X-ray treatment alone or of colchicine alone. All of the 89 rats classed as "no takes" in the experiments were later implanted with untreated tumor grafts and 63 proved to be "takes" showing that these animals were not constitutionally immune to cancer transplants. The average percentage of "takes" of the Flexner-Jobling carcinoma in our Madison strain of rats is about 85 as determined from over 3000 cancer transplantations. The infrequency of "takes" of implants which have been removed from colchicine-treated rats and rayed before implantation shows, therefore, that this treatment is highly lethal for such carcinomatous tissue.

In experiments with colchicine and X-rays on living rats bearing carcinomas the results, though not so striking, still show notable advantage of combined colchicine and X-ray treatment over X-rays

TABLE II.
Number of Takes of X-rayed (1500 *r*) Carcinoma Implants from Colchicine-treated Rats (0.2 mg per 100 g of body-weight) and of Similarly Rayed Carcinoma Implants from Rats Not Treated with Colchicine.

	Colchicine and X-ray	X-ray only	Colchicine only
Series XVI			
No. of rats used, 48	16	16	16
Results	Takes 2 No takes 14	Takes 15 No takes 1	Takes 14 No takes 2
Series XIX			
No. of rats used, 48	16	16	16
Results	Takes 6 No takes 10	Takes 10 No takes 6	Takes 11 No takes 5
Series XXX			
No. of rats used, 105	53	52	0
Results	Takes 9 No takes 44	Takes 46 No takes 6	

alone or colchicine alone, when moderate and frequent treatments with both X-rays and colchicine are employed.

It was not until some 20 series of experiments involving several hundred rats had been made that a significant difference was observed. In the earlier experiments even after the maximal toleration of X-ray and of colchicine dosage had been determined, there was heavy mortality among the treated animals and little to choose between the retardative or curative effects of combined X-rays and colchicine and those of X-rays alone as long as the irradiation dosage was as high as 1500 *r* or even 750 *r* a treatment for the 3 or 4 treatments given at intervals of about 2 weeks. Each kind of treatment, including colchicine alone, showed inhibitive effects and even cures but the differential benefits did not appear significantly between: (1) the combination of colchicine and X-rays; and (2) irradiation alone, until the rayings were reduced to 375 *r* a treatment and given at intervals of a week. The differences were still more marked when the irradiation was reduced to 188 *r* and given twice a week notwithstanding the increased hazards of such frequent anesthetization to the tumor-bearing rats.

Figure 1, for example, represents a graph based on our series XXIX, numbering 91 rats (32 receiving colchicine and X-ray treatment; 29 receiving X-rays only; and 30 receiving colchicine only). Treatment was begun 16 days after implantation when the tumors had reached a size of from 1 to 1.5 cm and were all actively growing. The colchicine dosage was 0.1 mg per 100 g of body weight given each time 15 hours before irradiation; rayings of 188 *r* were given each irradiated animal twice weekly. The perpendicular line represents size in centimeters, the horizontal, time in week intervals to

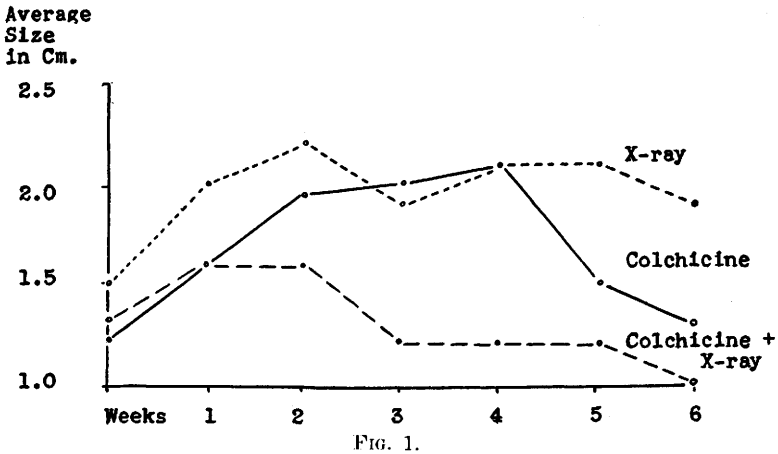

FIG. 1.

the end of the sixth week. Each week the tumors of all the surviving animals were measured (length by breadth) and the average for each group was recorded. From the graph it is evident that the tumors submitted to colchicine and X-ray treatment grew notably less on the average than those of either of the other classes. The graph for "colchicine only" is invalidated somewhat by the fact that 10 of the rats died one hot night, so that the later measurements were averaged on a smaller number of surviving individuals in this group. At the end of the sixth week 15 had been completely healed with 4 dead in the colchicine and X-ray group; 11 had been healed with 3 dead in the X-ray only group; and in the colchicine only group 8 were healed and 10 were dead.

Summary: X-rays were markedly more lethal to bits of carcinoma from colchicine-treated animals than to similar pieces of carcinoma from untreated animals, as shown by the infrequency of "takes" when implanted in susceptible animals. By administering colchicine to rats bearing actively growing carcinoma 15 to 18 hours before irradiation the effectiveness of X-rays in completely destroying or in retarding the growth of the cancer was increased. Light, frequent dosages of X-rays were less injurious to the rats and more effective in securing disappearance of the tumors than heavy, infrequent dosage.