ties as small as 5 to 10 cc could probably be used if the apparatus were produced in miniature. This would permit perfusion of an organ of a small rodent with blood from the same animal.

The plastic tubing imparts mobility to the apparatus and reduces the danger of breakage. In addition this tubing is nontoxic for organs as judged by its effect upon tissue cultured in close association with it. To test this fragments of 8-day chick embryo heart ventricles were planted in equal parts of blood plasma and embryo extract. Before clotting occurred 3 chips of tubing were set close to each tissue fragment so that if growth occurred it would be over and around the plastic. Of several plastics tested, "Voltron" was the only one which had no apparent effects over an observation period of 3 days. Preliminary to cultivating the tubing was sterilized by immersion in 70% alcohol for 24 hours and the alcohol was removed by immersion in sterile Tyrode solution.

Limitations of the Apparatus. Among the more serious limitations of the apparatus is the fact that when whole blood is used in considerable quantity the corpuscles settle in the reservoir. A means for agitating the fluid in the reservoir would be a desirable addition to this apparatus.

For sterile procedures the funnel has to be replaced by a more elaborate organ chamber. Suitable chambers have a drainage tube and an entrance for a 2-hole stopper. One opening in the stopper admits the cannula, the other a cotton filter through which escapes the oxygen which passed to the organ chamber from the reservoir.

The apparatus contains no filters. For non-sterile work we set a small pad of cotton over the neck of the funnel. If needed, more elaborate sand filters can be employed.

Still other refinements might be adopted, but the pump has proved to be satisfactory as it is. For example, an entire rat can be rendered blue by fluids containing various amounts of trypan blue. As another illustration, microscopic examination of fresh spreads or sections of tissues from a rat perfused with Ranvier's gelatin carmine showed complete injection of the vascular trees in the intestines, kidneys, liver, and other organs. Many but not all of the vessels in the skeletal muscles appear to be filled.

Summary. Description is given of an easily constructed perfusion pump and of the satisfactory results obtained with its use.

A type of plastic tubing used in this pump was found to have no discernible toxic effects in tissues cultured on it *in vitro*.

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Mitotic Response to Colchicine in Human Cancer.*†

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Colchicine, the alkaloid isolated from the corm and seed of *Colchicum autumnale* L, has been known to arrest nuclear divisions in the metaphase stage in generative tissue of plants and animals. During the past 15 years the studies with colchicine initiated by Dustin, his collaborators and students have been amply tested, and frequently reviewed (Levine[1] and Ludford[2]). Here, only references pertinent to the problem under investigation will be made.

Colchicine and about 47 other chemical

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† We are indebted to S. B. Penick and Co. through the courtesy of Dr. George M. Hocking for some of the colchicine used in this work.

1 Levine, M., *Bot. Rev.*, 1945, **11**, 145.

2 Ludford, R. J., *J. Nat. Cancer Inst.*, 1945, **6**, 89.

substances recorded by Krythe and Wellensiek[3] influence nuclear divisions in a similar manner. Yet the usefulness of colchicine, in preference to the many chemicals with this singular property, has been pointed out by many workers and its application to the study of endocrinology, genetics, and cytology is well established. Its use in cancer therapy has been studied on tumors in animals and while it is generally conceded that these growths are arrested by this drug, the effect is only temporary. Repeated doses of colchicine seem to produce cumulative effects and have proved toxic.

Roentgenologists have contended that dividing nuclei are more vulnerable to the action of X-rays than are the resting nuclei in a given tissue. Since colchicine arrests the dividing nucleus in metaphase, and causes an accumulation of cells in this phase, the combination of colchicine and X-ray suggested itself as a feasible therapeutic measure. These agents have already been combined in the treatment of animal tumors (see Levine[4]) and in several cases of human cancer as reported by Brücke and Heuber[5] and by Seed, Slaughter and Limarzi.[6] The results in animals seemed to indicate that this procedure has some value. In normal plant tissues (Levine[7]) one of us has shown that the application of small quantities of colchicine combined with X-irradiation of 900r or 1500r arrests growth. Neither agent, alone, produces this effect.

It is now well known that the optimum influence of a given dose of colchicine as determined by the number of arrested mitoses varies with the species of animal or plant treated. In the mouse and rat the maximum effect is produced in 9 to 14 hours. The poikilothermal animals studied show the largest number of arrested dividing nuclei at 160 to 180 hours after the administration of a given dose of colchicine (Delcourt 1939). In the onion root tip, the maximum number of metaphases after colchicine occurs at the 24th hour (Levine and Gelber[8]).

Similar studies of the effect of colchicine on human cancer cells have been few and incomplete, so that the period at which the maximum number of metaphases results after a given dose of the drug is unknown. Oughterson, Tennant and Hirshfeld[9] studied colchicine effects on a group of 21 cancer patients: 15 had control biopsies taken before receiving colchicine and 11 of these showed arrested mitoses in metaphase. Colchicine was administered after the control biopsy; this was followed in 9½ hours by another biopsy or in some cases by the removal of the entire tumor. In one case, biopsies were taken before administration of colchicine and at 5, 9½ and 12 hours after an injection of 4 mg of the drug. Twenty oil immersion fields were studied from each biopsy and mitoses were counted. The control showed 2.6 mitoses per field, while 5 hours after the injection 7.3 mitoses per field were counted. Specimens taken at 9½ hours yielded 12 mitoses, while the 12-hour specimens post-colchicine, gave 19.6 metaphases. These workers state that colchicine injections make it possible in some instances to obtain a more accurate index of the rate of growth of the tumor. Seed, Slaughter and Limarzi,[6] it seems, used colchicine for its toxic effects on the tumor. They used the drug after X-rays were administered. Brücke and Hueber[5] colchicinized 2 comparable tumors and irradiated one. These authors contend that X-rays must follow colchicine and must be applied in the karyokinetic crisis. However, they took no such precautions in the irradiation of their patient. There was no apparent consideration given to the cytological status of the tumors treated. The question as to when X-ray therapy should begin has

[3] Krythe, J. M., and Wellensiek, S. J., *Bibliog. Genet.*, 1942, **14**, 1.

[4] Levine, Michael, *Cancer Res.*, 1945, **5**, 107.

[5] Von Brücke, F. T., and Von Hueber, E. F., *Klin. Wchsch.*, 1939, **18**, 1160.

[6] Seed, L., Slaughter, D. P., and Limarzi, L. R., *Surgery*, 1940, **7**, 696.

[7] Levine, M., *Bull. Torrey Club*, 1945-1946, **72**, 563; **73**, 34; **73**, 167.

[8] Levine, M., and Gelber, S., *Bull. Torrey Bot. Club*, 1943, **70**, 175.

[9] Oughterson, A. W., Tennant, Robert, Jr., and Hirshfeld, John W., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 661.

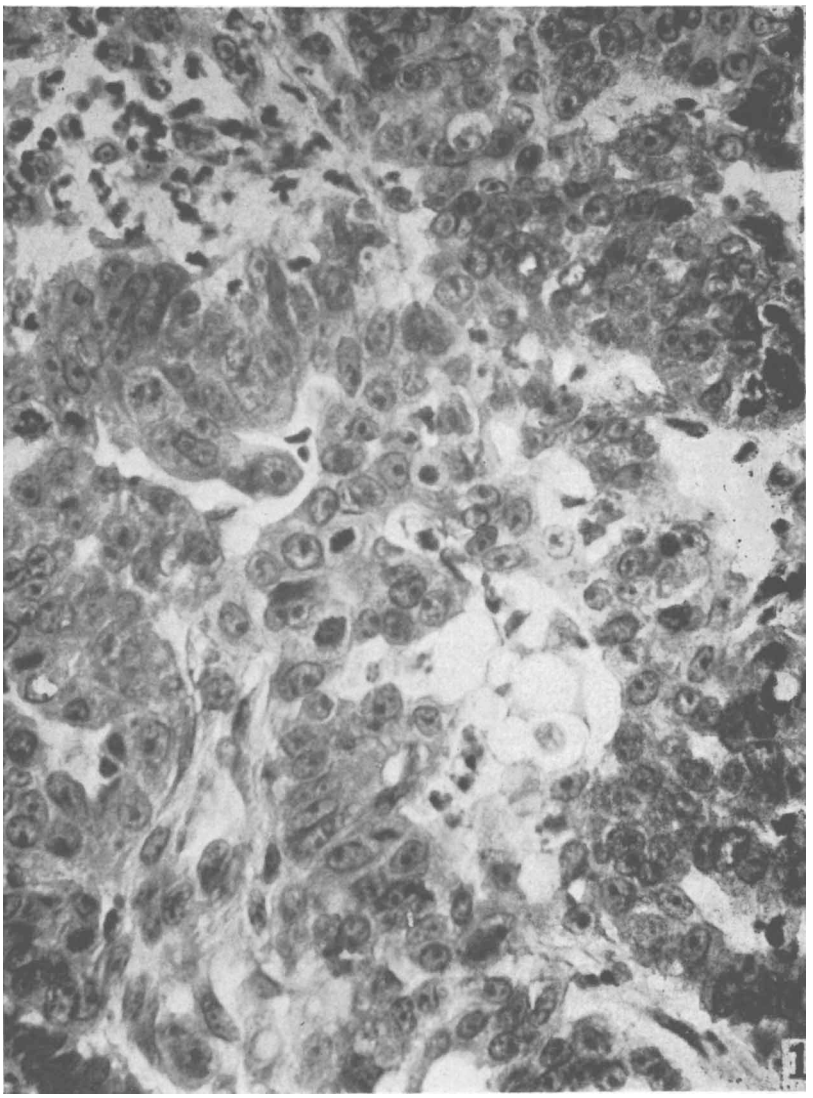

FIG. 1.
Case of B. G., photomicrograph of biopsy taken before the administration of colchicine. × 480.

not been considered. This appears significant in view of the contention that cells are most vulnerable to X-rays when the chromosomes are adequately exposed to these rays.

The following report deals with a study of the number of divisions (metaphases) in advanced carcinomas studied postoperatively following injections of 2 mg of colchicine. The primary purpose of this study is to determine as far as possible the time after colchicine administration when the greatest number of arrested mitoses appear in human cancer.

Methods and Materials. All the cases studied were postoperative, in terminal stages, with surface lesions. In preliminary studies on several of these patients mitotic counts were made at different times on different days without colchicine to verify the relative constancy of the number of division stages. No significant fluctuations were observed. After having made this observation, we studied the

TABLE I.
Average No. of Metaphases per H.P.F. Before and After 2 mg Colchicine.

Pt.	Diagnosis		Pre-colchicine	Post-colchicine in hr 4	8	12	16	20	24	32	48	72	96
BK	Ca bile duct		1.1	—	—	3.5	9.0	—	9.5	—	4.7	2.5	—
ML	Ca ovary		0.92	—	1.3	—	—	1.7	3.0	—	0.8	0.95	—
NT	Ca cervix		0.01	—	—	.01	2.2	0.8	0.1	—	0.1	0.6	—
JL	Epidermoid chin		0.0	0.4	0.8	1.0	0.8	1.7	1.2	—	1.4	0.9	—
CS	Ca rectum		0.72	0.9	1.7	1.1	1.2	—	3.0	—	1.0	1.2	0.45
RS	Ca sigmoid		1.3	5.5	5.2	13.4	—	14.3	13.8	5.9	1.3	1.4	—
YT	Epidermoid leg		1.1	1.2	2.0	1.6	2.1	2.2	2.2	1.8	3.9	4.7	2.3
				Partial Studies.									
BK	Ca bile duct	12/6/45	0.98				1.92						
		2/6/46	1.1				9.0						
BG	Ca sigmoid	3/12/46	6.1				21.4						
		3/19/46	5.6				39.0						

				Repeated doses of 2 mg colchicine at 24-hr intervals, followed by biopsy: 24 hr	48	72	96	120
MT	Ca cervix		0.43	2.1	2.0	3.2	3.7	1.6

response to 2 mg of colchicine introduced intramuscularly distant from the site of the neoplasm. Beginning at 4 hours after the colchicine injection, tissue was removed from the tumor at intervals of 4 hours for the first 24 hours and at daily intervals for the 2 days following. The tissues were fixed uniformly in Bouin's solution and imbedded in paraffin. Sections were cut 7½ μ in thickness and stained in Delafield's haematoxylin and eosin. A number of sections were mounted on each slide and the metaphases in 30 high power fields (H.P.F.) were recorded.

Observations. Of the 14 patients studied, 7 yielded suitable material for repeated counts, and the results are presented in Table I and composite graph. These were: one case of squamous cell carcinoma of the cervix, one adenocarcinoma of the rectum, one adenocarcinoma of the sigmoid, one carcinoma of the bile duct, 2 epidermoid carcinomas of the skin, and one adenocarcinoma of the ovary.

The metaphases counted in the precolchicinized tissue varied from zero to 1.3 per H.P.F. The first 10 hours after the treatment showed a slight increase in the mitotic count. Beyond this time the number of metaphases increased until a maximum was reached between the 16th and 24th hours. After that, there was a decline in the number of nuclei in this phase. At 72 hours after a single dose of colchicine, the count was still slightly greater than that at the beginning of the experiment. There was in general a uniform response to the colchicine as indicated in Table I with the maximum occurring most often at 24 hours after the injection. The number of cells that responded, however, varied considerably with the patient or the tumor and ranged from 1.7 to 14.3 per H.P.F. at this period. While the karyokinetic activity varied in number, 5 cases were proportionately similar. In 2 cases of the 7, B.K. and R.S., the number of metaphases was 10 times greater than in any of the other patients studied. The metaphase count in the case of Y.T. differed from all the other patients: starting from a value of 1.1 metaphases in the control biopsy, a first peak of 2.1 was reached at 16 hours, and a second one of 4.7 in 72 hours. The specimen obtained 96 hours after colchicine administration was still elevated and gave a count of 2.3 metaphases.

In the case of B.K. indicated in Table I

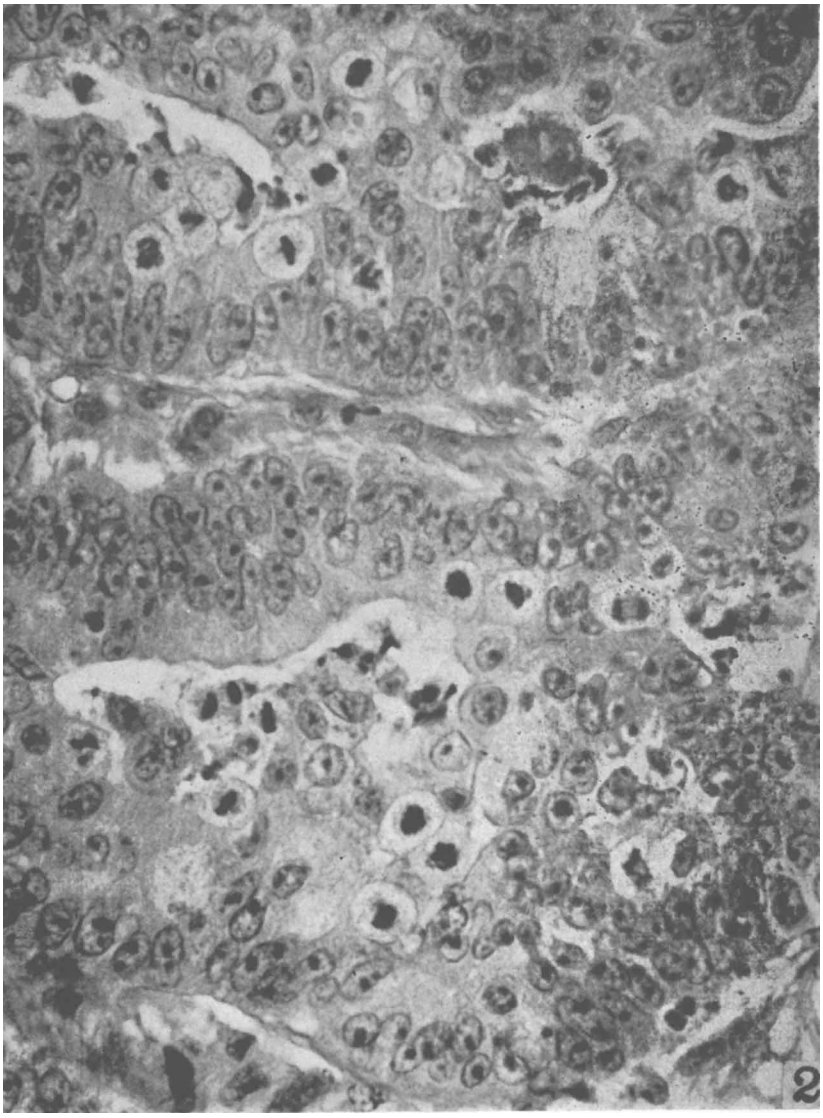

FIG. 2.
Same case, biopsy taken 7 days later, 16 hours after colchicine. × 480.

under "partial studies" 2 biopsies taken a day apart before administration of colchicine gave an average of 0.78 and 0.98 metaphases. Sixteen hours after colchicine the metaphase average increased to 1.92 per H.P.F. Two months later this patient was tested again: the control count revealed 1.1 divisions but at 16 hours after another injection of colchicine, the count rose to 9.0. Similarly in another case, B.G., the counts before and after 2 separate colchicine injections seemed significant. In this case the control biopsy made on March 12 gave an average of 6.1 metaphases (Fig. 1). After the injection of colchicine a second biopsy 16 hours later gave an average of 21.4 metaphases per H.P.F. Following a rest of 7 days, another biopsy from this case showed an average of 5.6 metaphases. This was followed by a second injection of a similar quantity of colchicine,

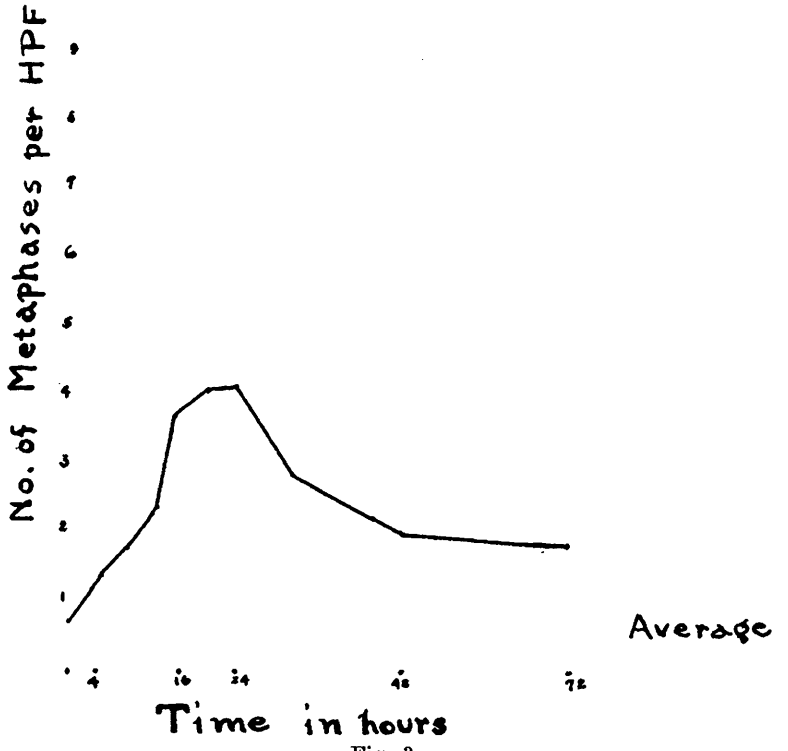

Fig. 3.

and 16 hours later another biopsy was done. The tissue showed an average of 39.0 metaphases per H.P.F. (Fig. 2).

It is of interest to note in both cases, B.K. and B.G., the second dose of colchicine gave a high metaphase count as compared with that found after an initial injection of the drug. The tumor tissue studied after a colchicine injection showed considerable hemorrhage and invasion of the tumor tissue by numerous white blood cells.

M.T., a case of cancer of the cervix with spread to the vulva, was biopsied at the site of the secondary tumor. The control section had very few division figures: no metaphases in 30 H.P.F. studied. The biopsy 12 hours after the injection of colchicine showed no change. The 16-hour biopsy had an average of 2.2 metaphases. Biopsies which followed at the 23rd, 44th and 69th hour showed no increase in the metaphase number. The small number of metaphases per field after colchicine seemed to indicate an exceedingly slow growing tumor and it appeared of interest to determine the influence on this tumor of repeated doses of this drug. After a rest of 30 days with the condition of the patient and the macroscopic appearance of the tumor unchanged, a biopsy was taken. This was followed by an injection of 2 mg of colchicine on 6 successive days. The results of these observations are likewise listed in Table I under "partial studies." The number of metaphases per H.P.F. prior to injection of colchicine was 0.43. The succeeding biopsy showed a rise to 2.1 metaphases per H.P.F. and after the 4th injection 3.7 metaphases were counted per H.P.F. This was followed by a decline to 1.6, 24 hours after the 5th injection. The microscopic preparations of this tissue contained extensive hemorrhage. The cancer tissue showed late mitotic stages.

Summary and Conclusions. 1. These observations seem to indicate that a single intramuscular injection of 2 mg of colchicine induces an arrest of nuclear division in the metaphase stage of human cancer. The number of cells in this stage increases slowly and the effect reaches a maximum between the

16th and 24th hours. The decline in number of metaphases is more gradual than the rise.

2. Repeated injections of colchicine in some patients after a relatively short rest period indicate a possible increased sensitivity to the drug.

3. Biopsies taken 48 to 72 hours after colchicine show an increase in hemorrhage, leucocytosis and some polyploid cells. Late telophases are occasionally observed in this colchicinized tissue.

4. The evidence presented here suggests the basis for further study of the effect of colchicine combined with other physical or chemical agents in the treatment of human cancer.

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Tantalum Oxide and Wound Healing: Experimental Study.

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Tantalum is a metallic element having an atomic weight of 180.88 and a density of 16.6. It has had some use in surgery, chiefly in the form of plates for substitution in cranial defects. It has been praised as being biologically inert.

Olson[1] reported that tantalum foil placed over wounds from industrial accidents where there had been partial loss of thickness of the skin seemed to produce epithelialization faster than any other method of treatment. It was assumed that this apparent stimulating action was due to the oxide coating of the tantalum which is always present. He then prepared a fine tantalum oxide powder and used it in treating small wounds and burns of the extremities. He reported that the acceleration of healing and the absence of pain were noteworthy. Since his studies did not include controls, we decided to do the following wound healing experiments.

Technic. The technic described by Brush and Lam[2] was used to test the effect of tantalum oxide powder on wound healing in 47 guinea pigs.

Under ether anesthesia the abdomen was shaved and treated with an antiseptic (hexylchloro-m-cresol). Using sterile scissors, an oval wound measuring approximately 8 x 10 mm in diameter and going through to subcutaneous tissue was made on each side of the midline. Fig. 1 illustrates the appearance of the wounds immediately after being made.

According to Carrel's law it is unnecessary for the wounds to be exactly similar because wounds in the same individual, even if of different size, tend to heal in the same period of time. The wound on the left of the animal was used to test the tantalum oxide, the right wound being the control. The outline of each wound was traced on sterile cellophane; then the tantalum oxide powder was placed on the left wound, and sterile gauze

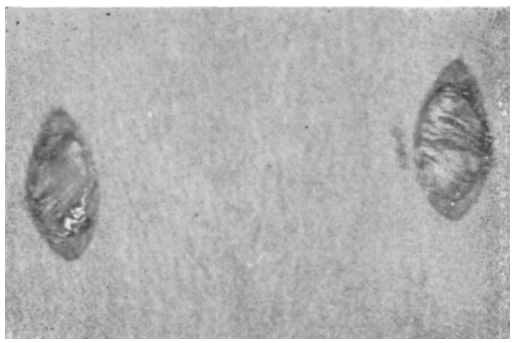

FIG. 1.
The appearance of the wounds just after being made.

[1] Olson, C. T., *Industrial Medicine*, 1945, **14**, 949.

[2] Brush, Brock E., and Lam, Conrad R., *Surgery*, 1942, **12**, 355.