ACTION OF COLCHICINE ON A TRANSPLANTED MALIGNANT LYMPHOID NEOPLASM IN MICE OF THE C3H STRAIN

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Colchicine is one of the alkaloids obtainable from the bulb, seeds, and leaves of Colchicum autumnale, Linnæus. This plant, commonly called "false saffron," "bastard saffron," "dog-killer," and "floating-light," is a bulbous liliaceous plant which blooms during the fall in the wet meadows of Central and Southern Europe, the leaves and seeds appearing the following spring. Its use as a quasi-specific remedy against the acute crises of gout dates back to ancient times, perhaps to the Byzantines. By these people, the Arabs, Salernitians, and others it was called "hermodact." This name, however, includes three botanical species: quintofolium, or long hermodact, Egyptian colchicum, or small hermodact, and Colchicum autumnale, which was imported from the East.

The first formula for this drug we owe to Jacques Psychriste, physician and counsellor of Emperor Leon the Great (457–475 A.D.). The physicians of that time prescribed it mixed with large doses of scammony or alternated with Persian pills of aloe, for they attributed evacuant and cathartic properties to the drug. The irritating effect on the digestive tract was to be soothed by aromatics and sweetmeats. Colchicum was declared to be deadly by Dioscorides and its use was abandoned for centuries.

The first scientific works on colchicum were contributed by Sennert (17th century), Stoerck (1763), Armstrong, Haden, Scudamore, Want and Williams (19th century). Colchicine was isolated by Houdé in 1887. It occurs in the form of a yellow powder or thin yellow flakes; it is odorless and more soluble in cold than in warm water. The aqueous solution is yellowish, levorotatory, unstable in the light, and very bitter to the taste. The alkaloid is soluble, also, in ethyl alcohol and in chloroform, but only slightly so in ether. It has no well defined melting-point and is decomposed by heat. Its empirical formula is given by Windaus (1924) as C₂₂H₂₅NO₆; its structural formula as

To quote from Fieser (13), it is a "phenanthrene alkaloid, for it has been degraded to a tetramethoxyxymethylphenanthrene and to 9-methylphenanthrene."

Physiological, pharmacological, and chemical studies of colchicine have been made by several investigators (Pelletier and Caventou, Bley, Winterstein, Jacobson, Dixon, Fühner and Rehbein, Jacobj, Beck, Coleman, Levison et al.). Dixon and Malden (5, 6) did the first cytological work in 1906 and 1908, but their observations were limited to the blood and bone-marrow. Their findings on leukocytosis were later corroborated by Beck, Lorthioir and Lits. Lits (15, 16) studied the general cellular reactions and lesions caused by the administration of the substance. Since 1934 many papers have been published, completing previous descriptions, giving various interpretations, and attempting to apply the latter.

The following is a summary of the findings of the Brussels school concerning the cytotoxicology of colchicine (16, 12, 4).

1. The blood of mice shows (a) a high and long-enduring polymorphonuclear leukocytosis, with the appearance of immature red and white cells and (b) slight variations in uric acid content.

2. In organs such as thymus, spleen, lymph nodes, and Peyer's patches waves of mitoses and pyknoses are found (Dustin's "caryoclastic shock").

3. Early or late mitotic hyperactivity can be seen in various tissues: the crypts of Lieberkuhn, granulosa cells of the ovarian follicles, germinal layers of epithelium of all types, hair follicles, lung, kidney, salivary and sebaceous glands, new growths, parenchymal cells, reticulo-endothelial cells, etc.

4. As a consequence of the variation of the mitotic activity in the digestive epithelium, an excretion of "nucleoid spherules" is present.

5. The alterations of the mitotic figures are typical, so that the latter receive the name "colchicinic mitoses." The dividing cells have a swollen appearance, clear cytoplasm, and clumped or scattered chromosomes belonging to the metaphase. Most of them die, but in certain circumstances a few may complete their normal cycle.

6. The reactions of the organism towards colchicine are independent of the route of administration, whether by injection, ingestion, or application in the form of an ointment.

7. The same effects upon mitosis are observed in cold-blooded animals and plants.

8. Aging of the solution of colchicine in water diminishes the activity of the drug, especially when the solution is exposed to light.

9. The mechanism of the action of colchicine in the acute crisis of gout is obscure and is not explainable by the cytological observations.

The effect of colchicine upon lymphatic tissue, and chiefly on the thymus, is not specific, for similar results follow the administration of other agents (16). Study of this group of substances was begun by Dustin in 1907, and since then has been carried on by him and his collaborators. These agents were termed "caryoclastic" and the chromatin material sensitive to them "euclastic." The theory of "differential susceptibilities" of various tissues was advanced as a result of these observations (8).

The similarity of the action of these nuclear poisons to that of x- and gamma radiations led Dustin to refer to their effect as "radiomimetic." It is quite logical, then, to test the action of colchicine upon tumors, especially a tumor composed of lymphoid elements, since normal lymphoid tissue is so
extremely sensitive to caryoclastic phenomena. The purpose of the present investigation is to describe the histology of a lymphoid tumor during regression induced by colchicine and determine whether the drug has a more selective propensity for the "malignant" than the normal lymphocyte.

**Effect of Colchicine on Tumors: Review of Literature**

Dominici (7) contributed the first empirical observations concerning the action of colchicine on tumors. He noted an improvement in the health of cancer patients treated with Colchicum autumnale.

Following the appearance of Lits' first paper (15), Dustin (9) studied the action of colchicine on a grafted sarcoma and presented the first scientifically controlled observations, indicating the possibilities of the use of the drug in the treatment of neoplastic growths. In a second series of experiments (10) he tested the action of the drug on Murray's guinea-pig liposarcoma and on tar cancer of the mouse. By biopsies he showed a threefold increase in mitotic activity in human carcinomas of the cervix when 2 mg. of colchicine were injected subcutaneously every day for three days. Oughterson, Tennant and Hirshfeld (18) observed a 700 per cent increase in the rate of mitosis in an adenocarcinoma of the sigmoid colon twelve hours after an intramuscular or subcutaneous injection of 1 to 4 mg. of colchicine. A similar action on the normal mucosa surrounding the tumor area was seen.

Lits (16) studied the effect of single and repeated administrations of colchicine upon mammary carcinoma C63. He showed that after one injection of colchicine the highest mitogenetic index in this carcinoma was 27.25 at twenty-four hours; the highest mitogenetic index in the Crocker sarcoma after colchicine administration was demonstrated by Dustin (10) as 15.7 at nine hours.

Amoroso (1) noted that the irradiated tumors of patients suffering from gout and being treated with colchicine decreased quickly and noticeably in size. He injected small amounts of colchicine every two days for two weeks into mice bearing grafted tumors. In two-thirds of the mice the tumors disappeared completely during this period of time, and in the remaining mice only small nodules remained. The histologic type of the tumor and the doses of colchicine are not recorded. A spontaneous epithelioma of the peritonsillar region in a dog regressed completely after forty days of treatment.

Peyron, Lafay and Kobozieff (19) and Peyron, Poumeau-Delille and Lafay (20, 21), using repeated treatments (injections and ointment applications) with colchicine, obtained histologically controlled regression of the Shope rabbit papilloma, the treated animals being immune to subsequent inoculations of the virus.

Ludford (17) was "unable to inhibit the growth of well established grafts of transplantable tumors with colchicine without the appearance of severe toxic..."
ACTION OF COLCHICINE ON A TRANSPLANTED MALIGNANT NEOPLASM

Clearkin (3) concluded that colchicine in the doses used had no inhibitory effect on the growth of mouse sarcoma S37 in vivo. "Growth in vitro of tumor obtained from an animal treated with colchicine was inhibited to a marked extent. . . . Sarcoma S37 is less affected by colchicine than many normal tissues."

Havas (14) detected a considerable inhibiting influence of colchicine upon the development of experimental tumors (crown-gall) in tomato plants. Finally, it is of interest to mention that E. Boyland and M. E. Boyland (2) found that colchicine in amounts approaching the toxic dose produced hemorrhage in grafted tumors accompanied by a reduction in their ascorbic acid content and metabolism.

MATERIALS AND METHODS

Mice of the strong C3H strain were used in the experiment to be described. A spontaneous case of leukemia from which transfers were originally made arose in a female which had received 3.9 mg. of equilin benzoate in 303 days. This mouse showed a bilobed mediastinal mass, whitish in color, adherent to the sternum. The spleen was enlarged. The long bones were solid and the pubis osteoporotic as a result of prolonged estrogenic treatment. Both thymic and splenic transplants grew in the first transfer generation but, since the grafted splenic tissue produced a better growth, the local tumor resulting at this implantation site was used for regrafting.5 From then on local tumor was used as donor tissue. Grafting was accomplished by inserting a small piece of tumor tissue subcutaneously into the right axilla, using a trocar for the purpose. The tumor at the implantation site usually grew to a considerable size (Fig. 1). The most satisfactory results were obtained when transplantation was done before any part of the tumor had become necrotic. The longer survival time in the ninth transfer generation (Table I) can be ascribed to the poor condition of the donor tissue.

Twenty-three days or more after implantation a general leukemic state was present in most animals. Spleen and lymph nodes were enlarged and there was leukemic infiltration in the kidneys, liver, bone marrow, etc. The white blood count was usually elevated, with a high percentage of lymphoid cells. In some instances a leukemic blood picture was not present, although there was a polymorphonuclear leukocytosis as a result of tumor necrosis. Just before death the tumor would decrease in size and the animal become very emaciated.

The growth was transplanted into 104 mice with no failures (13 transfer generations) and no spontaneous regressions. Starting with the fourth transplant generation (Series I) experiments were begun to determine the effect of colchicine on the tumor. The drug was administered subcutaneously. With Series I, 7/40 mg. in 0.5 c.c. distilled water was injected every second day for three times, no further treatment being given. Injections were begun after the tumor had been growing in the hosts for fourteen days. Additional injec-

5 Dr. W. U. Gardner kindly supplied the authors with this material in the third transplant generation. One hundred and seven animals have received tumor implants with "takes" in all cases. The growth is now in the 14th transfer generation. No tumor has regressed spontaneously.
Table I: Survival Time after Transplantation in Control and Colchicine-treated Animals
(Average Survival Time, Series I–IV: Controls 31.5 days; Colchicine-treated Animals 50.5 days)

<table>
<thead>
<tr>
<th>Controls</th>
<th>Colchicine</th>
<th>Transfer Generation</th>
<th>Age of Tumor when Transplanted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>32 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>29 &quot;</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>31 &quot;</td>
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<td></td>
<td>34 &quot;</td>
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<td></td>
<td>35 &quot;</td>
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<tr>
<td></td>
<td>22 &quot;</td>
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<td></td>
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<tr>
<td></td>
<td>28 &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Series I</td>
<td>40 days</td>
<td>40 days</td>
<td>T_1 to T_3 18, 31 and 28-day</td>
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<tr>
<td></td>
<td>42 &quot;</td>
<td>101 &quot; (killed)</td>
<td>growths</td>
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<tr>
<td>Series II</td>
<td>24 days</td>
<td>21 days</td>
<td>T_5 22 days</td>
</tr>
<tr>
<td></td>
<td>32 &quot;</td>
<td>28 &quot;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>34 &quot;</td>
<td>34 &quot;</td>
<td></td>
</tr>
<tr>
<td>Series III</td>
<td>25 days</td>
<td>57 days</td>
<td>T_4 22 days</td>
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<tr>
<td></td>
<td>28 &quot;</td>
<td>66 &quot; (killed)</td>
<td></td>
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<tr>
<td></td>
<td>33 &quot;</td>
<td>68 &quot; (killed)</td>
<td></td>
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<tr>
<td></td>
<td>36 &quot;</td>
<td></td>
<td></td>
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<tr>
<td>Series IV</td>
<td>26 days</td>
<td>44 days</td>
<td>T_7 19 days</td>
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<td></td>
<td>30 &quot;</td>
<td>45 &quot;</td>
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<td></td>
<td>30 &quot;</td>
<td>56 &quot;</td>
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<td>31 &quot;</td>
<td>56 &quot;</td>
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<tr>
<td></td>
<td>32 &quot;</td>
<td>57 &quot;</td>
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<td></td>
<td>35 days</td>
<td></td>
<td>T_8 26 days (old tumor; nec-</td>
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<td></td>
<td>37 &quot;</td>
<td></td>
<td>rosis accounts for long</td>
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<tr>
<td></td>
<td>39 &quot;</td>
<td></td>
<td>survival time)</td>
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<tr>
<td></td>
<td>42 &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>43 &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>31 days</td>
<td>15 animals killed</td>
<td>T_9 14 days</td>
</tr>
<tr>
<td></td>
<td>30 &quot;</td>
<td>for histologic</td>
<td></td>
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<tr>
<td></td>
<td>30 &quot;</td>
<td>study of tumor</td>
<td></td>
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<tr>
<td></td>
<td>25 &quot;</td>
<td>regression</td>
<td></td>
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<tr>
<td></td>
<td>31 days</td>
<td>following colchicine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 &quot;</td>
<td>administration</td>
<td></td>
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<tr>
<td></td>
<td>23 days</td>
<td></td>
<td>T_{10} 14 days</td>
</tr>
<tr>
<td></td>
<td>27 &quot;</td>
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<td></td>
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<tr>
<td></td>
<td>32 &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>32 &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>All animals</td>
<td></td>
<td>T_{12} 14 days</td>
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<tr>
<td></td>
<td>of T_{11} transfer generation killed for histologic study.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>23 days</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>30 &quot;</td>
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<tr>
<td></td>
<td>31 &quot;</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>33 &quot;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Animals of T_{11} and T_{14} transfer generations still alive.</td>
<td></td>
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</tr>
</tbody>
</table>

Doses were administered on the seventieth and seventy-sixth days after transplantation when a recurrence of the regressed tumor made its appearance at seventy days, in the animal of this group which survived 101 days after implantation of the tumor (Table I). The same dosage was used for the test animals of Series II, but only 2 injections were given.
In Series III and IV, where the best results on survival time were obtained, the same amount of colchicine ($\frac{3}{4}$ mg.) was dissolved in only 0.1 c.c. of distilled water, and injected subcutaneously every third day until the animal died or was killed. Injections were always made at a site far removed from the tumor. The mice used were young animals weighing 22–28 grams.

After it had been observed that tumors regress under the influence of colchicine therapy, a fifth series of animals was used for study of the histology of tumor regression and regeneration. Fifteen test animals of the ninth transfer generation comprised this group, with 4 additional tumor-bearing animals serving as controls. After the tumor had been growing fourteen days the first colchicine administration was made into all 15 test animals. Animals were killed eight, sixteen, twenty-four, forty-eight and seventy-two hours after the injection. At this time the 10 remaining animals were given a second dose, following which 5 animals were killed at the same time intervals as after the first dose. Seventy-two hours after the second injection the 5 remaining animals received a third dose of the drug, and were killed at similar intervals. Controls were killed fourteen, seventeen, twenty and twenty-three days after tumor implantation.

All test animals had a firm tumor about the size of a pea when colchicine
treatment was begun. Since central necrosis of these tumors does not set in until about the twenty-third day of growth the results cannot be attributed to spontaneous degenerative phenomena. Peripheral and central portions of the tumor and all lymphoid tissues were fixed in Dustin’s fluid and stained with Masson’s stain (modification with light green), which is especially effective for chromatin material following this fixation. Sections were cut at 5 micra, paraffin imbedding being used. Imprints of the tumor stained with Pappenheim’s combination of May-Grünwald and Giemsa solutions were also studied.

Observations

Following implantation of a few milligrams of the lymphoid tumor used, growth is relatively slow, but in fourteen days a solid mass the size of a large pea or kidney bean is present at the implantation site. Thenceforth the tumor increases in size more rapidly and by twenty-five days extends from the axillary to the inguinal fossa (Fig. 1). The time at which the mice begin to fail varies, the survival time differing accordingly. The central portion of the tumor becomes necrotic after twenty-three days, and before the animal dies there is usually general emaciation with a corresponding reduction in tumor size. After more than twenty days of tumor growth, leukemic infiltration of spleen, lymph nodes, bone marrow, kidneys and liver is observed. The histologic picture of these tissues is comparable to that in human lymphatic leukemia with loss of normal architecture in spleen and lymph nodes, periportal and sinusoidal infiltration in the liver, and intertubular lymphoid accumulations in the kidney. The white blood count is usually elevated (15–30,000 cells per cu. mm.), but not to the extreme degree observed in certain other lines of mouse leukemia, where counts of 300,000 or more have been observed by one of the authors (A. K.).

*For fixation and staining methods see p. 204.*
If $\frac{1}{40}$ mg. of colchicine is injected subcutaneously at a site far removed from a local fourteen-day growth of the transplanted tumor, the tumor mass does not continue to increase in size and loses its firm consistency. In some instances the tumor disappears within two days after the second injection, whereas in other animals the growth always remains palpable, although there is marked reduction in tumor size. Recurrence of the tumor may not be evident for two or three weeks (Fig. 2) and in one case no tumor recurred for five weeks. In no treated animal was there absolute suppression of tumor growth. When growth was suppressed it was usually for from two to three weeks. The tumor then resumed its former rate of growth and caused the death of the animal. The table gives data on survival time in untreated and colchicine-treated animals.

**FIG. 4. SECTION OF TUMOR SEVENTY-TWO HOURS AFTER THIRD COLCHICINE INJECTION**

The central portion shows reticular-cell stroma with a few well preserved lymphoid cells. The reticular cells proved to be more resistant than the lymphoid cells to the action of colchicine. The cells in the periphery are for the most part pyknotic. $\times 100$.

Histologically the fourteen-day growth of transplanted tumor is a compact mass of lymphoid cells with a supporting reticular cell framework (Fig. 5). Mitotic figures are numerous and there is pyknosis of some nuclei.

**Microscopic Changes Following Colchicine Administration**

**One Injection (animals killed fourteen to seventeen days after transplantation of tumor): Eight Hours after Injection**

**Tumor:** Mitotic activity has increased and there is much more nuclear pyknosis (Fig. 6) than in the untreated tumor.

**Spleen and Lymph Nodes:** Colchicine mitoses and pyknotoses in germinal centers, the degree of nuclear pyknosis being less than in the tumor.

**Thymus:** Numerous colchicine mitoses but no pyknosis in the cortex.

**Sixteen Hours after One Injection**

**Tumor:** Extreme degree of pyknosis and colchicine mitoses in the metaphase showing clumped chromatin (Fig. 7).
FIGURES 5-16

Photomicrographs X 400. Sections cut at 5 micra. Tissue fixed in Dustin's fluid and stained with Masson stain.

FIG. 5. FOURTEEN-DAY GROWTH OF TUMOR: CONTROL, UNTREATED BY COLCHICINE

FIG. 6. TUMOR EIGHT HOURS FOLLOWING ONE INJECTION OF COLCHICINE (1/40 MG.)
Note pyknoses and colchicinic figures (caryochlastic shock).

FIG. 7. TUMOR SIXTEEN HOURS FOLLOWING ONE INJECTION OF COLCHICINE: INCREASED DEGREE OF REACTION SHOWN IN FIG. 6

FIG. 8. TUMOR TWENTY-FOUR HOURS FOLLOWING ONE INJECTION OF COLCHICINE
Note loosely arranged structure of tumor and extreme pyknosis.

FIG. 9. TUMOR FORTY-EIGHT HOURS FOLLOWING ONE INJECTION OF COLCHICINE: RECOVERY PHASE; MORE COMPACT STRUCTURE; FEW PYKNOTIC CELLS

FIG. 10. TUMOR SEVENTY-TWO HOURS FOLLOWING ONE INJECTION OF COLCHICINE: REGENERATION MITOSES; SAME GENERAL STRUCTURE AS CONTROL TUMOR

FIG. 11. SEVENTEEN-DAY GROWTH OF TUMOR: CONTROL, UNTREATED BY COLCHICINE; SAME STRUCTURE AS IN FIG. 5

FIG. 12. TUMOR EIGHT HOURS FOLLOWING SECOND INJECTION OF COLCHICINE (INTERVAL OF SEVENTY-TWO HOURS BETWEEN INJECTIONS)
Note more pyknosis and greater disorganization than in Fig. 6.

FIG. 13. TUMOR SIXTEEN HOURS FOLLOWING SECOND INJECTION OF COLCHICINE

FIG. 14. TUMOR TWENTY-FOUR HOURS FOLLOWING SECOND INJECTION OF COLCHICINE

FIG. 15. TUMOR FORTY-EIGHT HOURS FOLLOWING SECOND INJECTION OF COLCHICINE
Compare with Fig. 9. Structure of tumor is less dense and shows more pyknosis than forty-eight hours after one injection.

FIG. 16. TUMOR SEVENTY-TWO HOURS FOLLOWING SECOND INJECTION OF COLCHICINE: LOSS OF ARCHITECTURE OF TUMOR ALTHOUGH RECOVERY HAS TAKEN PLACE

Fixation of Tissue in Dustin's Fluid

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% Chromic acid</td>
<td>75 parts</td>
</tr>
<tr>
<td>40% Formaldehyde</td>
<td>20 parts</td>
</tr>
<tr>
<td>Glacial acetic acid</td>
<td>5 parts</td>
</tr>
</tbody>
</table>

Fixative prepared immediately before use.

Staining

After removal of paraffin the sections are placed in:

1. Ferric alum 2-3% .............. 8 to 24 hours (35° C.)
2. Ferric hematoxylin ............ 8 to 24 hours (0.5% aq. sol.)
3. Differentiation under microscopic control in ferric alum 5-10%.
4. Wash in distilled water.
5. Stain in
   Red ponceau de xylidine ........... 1.8 gr.
   Acid fuchsin ................................ 0.2 gr.
   Glacial acetic acid ................ 2. c.c.
   Distilled water ......................... 200 c.c.
   30 seconds to 5 minutes.
6. Wash quickly in distilled water.
7. Differentiation (not under microscopic control) in
   Phosphomolybdic acid ............... 2 gr.
   Distilled water .................... 200 c.c.
   15 minutes to 4 hours (as long as possible).
8. Without washing, put directly into
   Light green .......................... 2 g.
   Glacial acetic acid ................ 2 c.c.
   Distilled water .................... 200 c.c.
   30 seconds to 5 minutes.
9. Wash in
   Distilled water .................... 200 c.c.
   Glacial acetic acid ................ 2 c.c.
10. Alcohols, xylol, etc.
Spleen and Lymph Nodes: Same picture as at eight hours, with greater nuclear degeneration in dividing cells.

Thymus: Pyknosis and mitosis in the larger, round (blastic) and reticular cells (Fig. 28).

Twenty-four Hours after One Injection

Tumor: Extreme degree of pyknosis, reticular cells more apparent, very few mitoses, destruction of compact tumor architecture (Fig. 8).

Spleen and Lymph Nodes: Normal structure with a few more mitoses than can be seen in the normal organ.

Thymus: Slight inversion.5 A few mitoses and pyknoses in blastic cells. Phagocytic activity.

Forty-eight Hours after One Injection

Tumor: Dense structure again, but not as compact as untreated tumor. Slight increase in mitotic activity with mitoses abnormal (Fig. 9).

Spleen and Lymph Nodes: Normal structure.

Thymus: Reconstruction of cortical structure under way. Large phagocytes containing digested material. No pyknoses. Mitoses necessary for reconstruction process evident.

Seventy-two Hours after One Injection

Tumor: General aspect of untreated tumor; more mitoses (not colchicinic), however, and tumor not quite so compact (Fig. 10).

Spleen and Lymph Nodes: Normal.

Thymus: Approaching normal.

Control

Untreated Seventeen-day Tumor: Same picture as fourteen-day untreated growth. No necrotic areas (Fig. 11).

Two Injections (animals killed seventeen to twenty days after transplantation of tumor): Eight Hours after Second Injection

Tumor: Numerous colchicinic mitoses (metaphases and prophases). Many more mitotic figures than eight hours after one injection (compare Figs. 6 and 12).

Spleen and Lymph Nodes: Mitoses in germinal centers. Same picture as eight hours following single injection.

Thymus: Same general effect as eight hours after one injection, but quantitatively fewer cells in cortex since cortex was not completely rebuilt (Fig. 29). Colchicinic mitoses present.

Sixteen Hours after Second Injection

Tumor: More mitoses than after eight hours. Some cells in colchicinic mitosis. Architecture similar to that described twenty-four hours after one injection. Tumor not compact (Fig. 13).

Spleen and Lymph Nodes: Same reaction as after one injection.

Thymus: Same reaction as sixteen hours after one injection, but fewer pyknotic nuclei seen in each field since the cortex had not been rebuilt.

Twenty-four Hours after Second Injection

Tumor: Most of the nuclei pyknotic. Loose structure (Fig. 14).

5 The term "inversed thymus" indicates clear or light cortex and dense medulla, the reverse of the normal situation.
Spleen and Lymph Nodes: Normal.
Thymus: Pyknosis in cortex of “inversed” thymus.

Forty-eight Hours after Second Injection
Tumor: Denser structure of tumor than after twenty-four hours. Recovery phase (Fig. 15). Some evidence of phagocytosis.
Spleen and Lymph Nodes: Normal.
Thymus: Inversed. No pyknosis.

Seventy-two Hours after Second Injection
Tumor: Mitotic activity similar to seventy-two hours after one injection, but structure of tumor less dense (Fig. 16).
Spleen and Lymph Nodes: Normal.
Thymus: Inversed.

Control: Untreated Twenty-day Tumor: Same structure as untreated fourteen-day and seventeen-day growths (Fig. 17). No necrosis. Spleen and lymph nodes of animal bearing this tumor not leukemic.

Three Injections (animals killed twenty to twenty-three days after transplantation of tumor): Eight Hours after Third Injection
Tumor: Same general effect as eight hours after second injection. Mitoses more pyknotic, however, and absolute number of cells affected fewer, since tumor was more diffuse at time of injection (Fig. 18).
Spleen and Lymph Nodes: Reaction same as eight hours after one injection.
Thymus: Inversed. Pyknosis of larger cells of cortex. Reticulum prominent (Fig. 30).

Sixteen Hours after Third Injection
Tumor: Pyknosis and phagocytosis. More cells affected than at eight hours (Fig. 19).
Spleen and Lymph Nodes: Reaction same as sixteen hours after one injection.
Thymus: Inversed. Depleted of round cells.

Twenty-four Hours after Third Injection
Tumor: Large areas of necrosis, much pyknosis and disorganization of tumor (Fig. 20). Some islets of resistant round cells (Fig. 26).
Spleen and Lymph Nodes: Normal.
Thymus: Inversed, with some pyknosis.

Forty-eight Hours after Third Injection
Tumor: Reticular cells predominate, with very few lymphoid cells present (Fig. 21). Most of the remaining lymphoid cells pyknotic; phagocytosis of pyknotic elements. Large necrotic areas. Mitoses in reticular cells (Fig. 3).
Spleen and Lymph Nodes: Normal.
Thymus: Inversed. No pyknosis.

Seventy-two Hours after Third Injection
Tumor: Almost entirely necrotic (Fig. 4). Reticular framework remains. Mitosis in reticular cells. Some areas show nests of lymphoid cells as in Fig. 26.
Spleen and Lymph Nodes: Normal.
Thymus: Inversed.
FIGURES 17–27

Photomicrographs × 400. Sections cut at 5 micra. Tissue fixed in Dustin's fluid and stained with Masson stain.

FIG. 17. Twenty-day Growth of Tumor: Control, Untreated by Colchicine; Same Structure as in Figs. 5 and 11

FIG. 18. Tumor Eight Hours Following Third Injection of Colchicine, Given Seventy-two Hours After Second Injection: Extreme Pyknosis and Cellular Degeneration

FIG. 19. Tumor Sixteen Hours Following Third Injection of Colchicine: Pyknosis and Degeneration

FIG. 20. Tumor Twenty-four Hours Following Third Injection of Colchicine: Most of Round Cells Pyknotic; Intact Reticular Cells

FIG. 21. Tumor Forty-eight Hours Following Third Injection of Colchicine: Mitoses in Reticular Cells; Relatively Few Lymphoid Cells, and Those Present Pyknotic

FIG. 22. Tumor Seventy-two Hours Following Third Injection of Colchicine: Reticular Cells Predominant; Very Few Lymphoid Cells

FIG. 23. Twenty-three-Day Growth of Tumor: Control, Untreated by Colchicine; Same Structure as in Figs. 5, 11 and 17

FIG. 24. Section of Recurrent Tumor (101 Days after Implantation, 87 Days after First of Five Colchicine Injections); Same Structure as Untreated Tumor (81 Days after the Primary Regression)

FIG. 25. Spleen of Animal Bearing Tumor Shown in Fig. 24: Leukemic Structure, Similar to Transplanted Tumor Used in this Experiment

Colchicine treatment of the local tumor did not prevent the appearance of systemic leukemia.

FIG. 26. Islet of Resistant Lymphoid Cells in Pyknotic Area, from Section of Tumor Twenty-four Hours after Third Injection of Colchicine

FIG. 27. Pyknosis of Lymphoid Cells Infiltrating Abdominal Muscles, from Section Taken Sixteen Hours after Third Injection of Colchicine

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FIGURES 28-32

Photomicrographs × 400 (except Fig. 31). Sections cut at 5 micra. Tissue fixed in Dustin’s fluid and stained with Masson stain.

Fig. 28. Thymic Cortex Sixteen Hours after One Injection of Colchicine, Same Animal as Fig. 7

Note pyknosis of lymphocytes.

Fig. 29. Thymic Cortex Eight Hours after Second Colchicine Injection: Pyknosis of Lymphocytes; Same Animal as Fig. 12

Fig. 30. Thymic Cortex ("Inversed Thymus") Eight Hours after Third Colchicine Injection: Reticular Cells Prominent; Remaining Round Cells Pyknotic. Cortex Practically Devoid of Lymphocytes; Reaction Similar to That Shown by Tumor in Fig. 20

Fig. 31. Spleen Seventy-two Hours after Third Colchicine Injection: Normal Architecture; Same Animal as Fig. 22

Splenic tissue is resistant to colchicine in the doses used in this experiment. × 100.

Fig. 32. Spleen (Same Section as Fig. 31, But Higher Magnification), Showing Normal Malpighian Corpuscle

Splenic and lymph node lymphocytes are not destroyed by colchicine in contrast to the malignant lymphoid cells of the transplanted tumor (3 injections 1/10 mg. colchicine spaced seventy-two hours apart).
Control

Untreated Twenty-three-day Tumor: Similar structure to other sections of untreated tumor (Fig. 23). Grossly the central portion of this tumor was necrotic, the periphery healthy.

Tumor Recurring after Colchicine Administration

A compact lymphoid tumor has formed (Fig. 24).

Discussion

Since a local transplanted lymphoid tumor grew for a period of three weeks without the entire animal becoming leukemic, it was possible to compare the effect of colchicine on the malignant lymphoid cells and normal lymphocytes in the same animal. It appears that the lymphocytes of the tumor become increasingly susceptible to the action of colchicine, from the standpoint both of number of cells affected and the time it takes for the action to manifest itself. Normal lymphocytes, on the other hand, as has already been demonstrated, become neither more nor less sensitive to the so-called "caryoclastic agent" (16). After the first and second injections (spaced at seventy-two hour intervals) recovery of the tumor is almost complete, there being, however, a more marked reaction to the second injection. There are certain changes in the architecture of the tumor, which has a less compact structure than the untreated growth. Following the third injection the tumor rapidly disintegrates, with pyknosis of most of the lymphocytes, a reticular framework remaining. The reticular cells appear to be more resistant than the round elements and it is from the former that the recurring tumor is probably rebuilt (Fig. 3), although islets of resistant lymphocytes remain (Fig. 26).

Colchicine, as used in this experiment, cannot be considered curative, for in every instance there was recurrence of treated tumors, even if growth was suppressed for as long as five weeks. This alkaloid is, however, a substance whose harmful action is more selective for the malignant than for the normal lymphocyte, the lymphocytes of the thymus excepted (Figs. 28, 29, 30). Proof of this statement lies in the fact that after three injections there was complete disintegration of the tumor, whereas the spleen and lymph nodes of the same animals retained their normal structure (Figs. 31 and 32).

Failure to obtain regression of colchicine-treated sarcomas in the experiments of Clearkin and Ludford can be explained by the theory of "differential susceptibilities" proposed by Dustin (8). One of the authors (F. J. L.) has already demonstrated that some tumors are more susceptible than others to the action of the drug (16). Since the lymphocyte is the cell most susceptible to the action of colchicine in the normal animal, it is not surprising that the lymphoid tumor studied here responded so well.

It appears that the reticular cells are more resistant than the lymphoid cells to colchicine. Although a series of tumors at different stages in recurrence are necessary to prove the point, it appears from this investigation that regeneration of lymphoid tumors can proceed from reticular cells.
Summary and Conclusions

1. A malignant transplanted lymphoid tumor was subjected to the action of repeated subcutaneous injections of $\frac{1}{10}$ mg. colchicine in 0.1 c.c. distilled water every three days, injections being made at some distance from the tumor.

2. Regression of the tumor was brought about by this treatment and the animals survived 50.5 days after implantation as compared with 31.5 days for untreated controls (13 controls and 14 test animals).

3. Histologic studies showed that regression was due to repeated so-called "caryoclastic shocks." The lymphocytes of the tumor became pyknotic and died.

4. The reticular cell elements of the tumor were most resistant to treatment by colchicine. There were also some foci of resistant lymphocytes. Tumors which recurred seemed to arise by proliferation of reticular cells of the regressed growth. Both the lymphoid and reticular cells of the lymphosarcomatous growth studied may be considered malignant.

5. A differential susceptibility to colchicine exists between normal and malignant lymphocytes, the malignant cells being much more susceptible. Normal thymic lymphocytes are destroyed quite readily by the drug in contrast to the more resistant splenic and lymph node lymphocytes.

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Bibliography