STUDY ON THE ANTICANCEROUS ACTION OF UNSATURATED SEVEN MEMBERED RING STRUCTURE, ESPECIALLY COLCHICINE DERIVATIVES

(With Plates I and II)

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INTRODUCTION

Colchicine has been used since an early period of medical history. Knowledge of its usage and nature may be divided into three stages. In the first stage, which may be called the classical stage, it was thought to be a placebo for gout and its alkaloid present in the corm, seeds and flowers of Colchicum autumnale was used. The second stage began with the reports that it was poisonous agent against cell division by Pernice\(^1\), Amoroso\(^2\) and Lits\(^3\). Thereafter, many papers on experimental and clinical subjects about colchicine were published. However, since colchicine was very poisonous, it was not a successful agent for the purpose of chemotherapy for human cancer. The third stage began with the study of the effect of colchicine-like substances. Brues and Cohen\(^4\) reported the effect of 10 colchicine-like substances on regenerating liver in experimental animals. In 1945, Dewar\(^5\) rewrote the formula of colchicine (I) which was proposed by Windaus\(^6\), claiming that colchicine possessed unsaturated seven membered ring structure. In 1950, Nozoe\(^7\), Doering\(^8\), Cook\(^9\) and Haworth\(^10\) were successful in synthesizing tropolone (II), independently. Subsequently it was confirmed that colchicine has a chemical formula shown in (III). The effect of many colchicine derivatives were reported by Santavy\(^11\), Ullyot\(^12\) and Leiter et al.\(^13\)\(^14\). On the other hand, Katsura and his associates\(^15\)\(^16\)\(^17\) carried out experiments on many tropolone derivatives, especially studies on C ring structure were performed. According to these studies, tropolone was found to have no effect by itself but tropolone methyl ether (IV) had slight colchicine-like effect in the lethal doses, while its action was much inferior to colchicine. Sato\(^18\), disclosed that it was hard to obtain the so-called colchicine-like effect with a single ring compound of tropolone in his experiment. Therefore it may be significant to test the effect of poly-membered ring structure including tropolone and new colchicine derivatives on tumor cells.

[15]
EXPERIMENT (1)

Screening Test on Yoshida Sarcoma

The effect of 20 compounds (Fig. 1. and Table 1. III, V-XXIII) with unsaturated seven membered ring structure, which are chiefly colchicine derivatives, on Yoshida sarcoma was studied. The technique and assay of the effect on sarcoma cells were those of Yoshida's screening test method, and the aceto-gentian violet was used for the stain. The administered concentration was studied in many cases.
in dosage levels of 5 mg, 2 mg, 1 mg, 0.5 mg, 0.2 mg, 0.1 mg, and so on per 100 g of body weight.

Table 1.

<table>
<thead>
<tr>
<th>(I)</th>
<th>Colchicine (Windaus)</th>
<th>Vehicle</th>
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<tbody>
<tr>
<td>(II)</td>
<td>Tropolone</td>
<td>p. g.</td>
</tr>
<tr>
<td>(III)</td>
<td>Colchicine</td>
<td>water</td>
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<tr>
<td>(IV)</td>
<td>Tropolone methyl ether</td>
<td>p. g.</td>
</tr>
<tr>
<td>(V)</td>
<td>Trimethylcolchicinic acid methyl ether (a)</td>
<td>water</td>
</tr>
<tr>
<td>(VI)</td>
<td>Thiocolchicine</td>
<td>p. g.</td>
</tr>
<tr>
<td>(VII)</td>
<td>Thiocolchicine</td>
<td>p. g.</td>
</tr>
<tr>
<td>(VIII)</td>
<td>Condensation product of colchicine-hydrazid with acetone</td>
<td>p. g.</td>
</tr>
<tr>
<td>(IX)</td>
<td>Cadensation product of colchicine-hydrazid with methyl ethyl ketone</td>
<td>p. g.</td>
</tr>
<tr>
<td>(X)</td>
<td>Colchineamid</td>
<td>p. g.</td>
</tr>
<tr>
<td>(XI)</td>
<td>Condensation product of colchicine with guanidine</td>
<td>p. g.</td>
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<tr>
<td>(XII)</td>
<td>S-ethyl thiocolchicine</td>
<td>p. g.</td>
</tr>
<tr>
<td>(XIII)</td>
<td>5-Amino-4-(β-hydroxy-γ-phenyl propyl) tropolone triacetate</td>
<td>p. g.</td>
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<td>(XIV) 5-Amino-4-(β-hydroxy-γ-phenyl propyl) tropolone</td>
<td>p. g.</td>
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<td>(XV) 2-Amino-1,3-diazazulene</td>
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<td>(XVI) 2-Hydroxy-1,3-diazazulene</td>
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<td>(XVII) β-Trimethoxy-styryl-tropolone</td>
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<td>(XVIII) 3-(p-tolyl)-sulfonyl amino tropolone</td>
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<td>(XIX) 2-Amino-5-isopropyl-1, 3-diazazulene</td>
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<td>(XX) 2-Mercapto-1, 3-diazazulene Na salt</td>
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<td>(XXI)5-Amonotropolone HCNS salt</td>
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<td>(XXII) Mercaptotropone</td>
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<td>(XXIII) Diaminotroponimine HCl salt</td>
<td>water</td>
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</table>

Indication: (*) d-tartrate derivative used.
p. g. propylene glycol

Those which showed influence on the tumor cells were as follows.
1) Colchicine (III).

Scattered diplochromosomes which were observed one hour after subcutaneous injection of 0.1 mg (approximately 1/2 M.L.D.) (Fig. 2.) of this substance reached the maximum at the 6th hour, and occupied most of the accumulated metaphase. Though the accumulation of metaphase reached the maximum at the 12th hour, scattered diplochromosomes did not increase after the 6th hour, and abnormal
metaphase such as the so-called star metaphase or distorted star metaphase, ball
metaphase, etc., increased. Though these changes could be observed at the 24th
hour, scattered diplochromosomes could hardly be seen. Ana- and telophase which
did not appear up to 12 hours after injection started to appear at the 24th hour.
In the resting tumor cells, the extracellular outflow of cytoplasm was observed
1-3 hours after injection, which decreased with lapse of time and was replaced by
remarkable increase of caryolysis, caryorexis, etc. In cases of subcutaneous
injection of 0.05 mg (approximately 1/4 M.L.D.), colchicine-mitose were slightly
observed at the 3rd and 6th hour, while ana- and telophase did not disappear.

2) Trimethylcolchicinic acid methyl ether d-tartrate (T.M.C.A. methyl ether
d-tartrate) (V)\(^{12,13}\)

With subcutaneous injection of 2 mg (approximately 1/2.5 M.L.D.) (Fig. 3.) of
this substance, the destruction of metaphase which appeared from one hour after
injection started to accumulate from the 6th hour, and reached the maximum at
the 12th hour. At this time scattered diplochromosomes and necrobiotic metaphase
were both conspicuous. At the 24th hour no scattered diplochromosomes were
observed, which were replaced with increasing rate of necrobiotic metaphase.
Ana- and telophase which were not observed till the 12th hour after injection
started to appear slightly after the 48th hour, and normal mitoses proceeded.
Only slight prolongation of life was observed against controls without injections.
With subcutaneous injection of 1 mg of this substance (approximately 1/5 M.L.D.)
(Fig. 4.), accumulations of normal and abnormal metaphase were both at the
maximum at the 12th hour, but scattered diplochromosomes were seen at most
at the 6th hour. The findings of destruction became little at the 24th hour.
Ana- and telophase both started to disappear one hour after injection, and at the
12th hour abnormal ana- and telophase which showed marked difference of number of chromosomes despite division into dipolar, or picture with polypolar appeared. With subcutaneous injection of 0.5 mg (approximately 1/10 M.L.D.) (Fig. 5.), the whole picture followed that of 1 mg in general, but the degree of destruction was slight. Abnormal metaphase which was seen up to 12 hours disappeared at the 24th hour. In case of subcutaneous injection of 0.2 mg of this substance (approximately 1/25 M.L.D.) (Fig. 6.), the effect markedly decreased, and although scattered diplochromosomes were observed up to the 6th hour, it was 1%-2% of the tumor cells. Ana- and telophase began to recover at the 6th hour, and
returned to normal by the 12th hour. With subcutaneous injection of 0.1 mg (approximately 1/50 M.L.D.) (Fig. 7.), abnormal metaphase was observed up to the 6th hour. Scattered diplochromosomes were, however, seen at most at the first hour and only few were seen 3 hours later. Ana- and telophase both recovered completely to normal at the 6th hour. With subcutaneous injection of 0.05 mg

Fig. 8. V, f.

Trimethylcolchicinic acid methyl ether d-tartrate 0.05 mg/100 g subcutaneous injection.

Fig. 9. V, g.

Trimethylcolchicinic acid methyl ether d-tartrate 0.02 mg/100 g intraperitoneal injection.
(approximately 1/100 M.L.D.) (Fig. 8.), accumulation of abnormal metaphase was slight. Most of them were star metaphase. Ana- and telophase disappeared within 1-3 hours which was followed with recovery to normal. No influence was observed with subcutaneous injection of 0.02 mg (approximately 1/250 M.L.D.). Destruction of resting cells was observed remarkably with dosage of 2 mg, followed with slighter effect with decrease of quantity.

In case of intraperitoneal injection, some influence was seen with 0.02 mg (approximately 1/250 M.L.D.) (Fig. 9.), while the effect continued up to 12 hours with dosage of 0.2 mg (Fig. 10.). Scattered diplochromosomes were very slightly seen one hour after the injection with 0.05 mg. Ana- and telophase began to appear by the 12th hour with 0.1 mg, by the 6th hour with 0.05 mg, and by the 3rd hour with 0.02 mg.

3) Thiocolchicine (VI).20)21)

Colchicine-like effect which appeared an hour after subcutaneous injection of 0.2 mg of this substance (approximately 1/2.5 M.L.D.) (Fig. 11.) became remarkable at the 12th hour and a relatively larger number of metaphase, which was seen in about 30%-40% of the tumor cells, was taken over by the scattered diplochromosomes. The above changes lessened after 24 hours, and accumulation of normal metaphase, distorted star metaphase and necrobiotic metaphase appeared mixed with remark-
ably destroyed and necrotic tumor cells. After 48 hours, abnormal division of tumor cells was hardly seen. The overall picture caused by subcutaneous injection of 0.1 mg of this substance (approximately 1/3 M.L.D.) (Fig. 12.) showed lesser changes than that of 0.2 mg. It, however, followed almost the same course, and 12 hours later, scattered diplochromosomes accumulated in a rather large number (Plate I, Fig. 1). The distorted picture which was seen at the 24th hour started to recover with the appearance of ana- and telophase at the 48th hour. After subcutaneous injection of 0.05 mg (approximately 1/10 M.L.D.) (Fig. 13.), scattered diplochromosomes appeared 3-6 hours later. The accumulation of metaphase which appeared at the 6th hour mostly consisted of normal metaphase and

![Graphs showing mitotic cells over time for different doses of thiocolchicine.](image_url)

**Fig. 13.** VI, c. Thiocolchicine 0.05 mg/100 g subcutaneous injection.

**Fig. 14.** VI, d. Thiocolchicine 0.01 mg/100 g intraperitoneal injection.

**Fig. 15.** VI, e. Thiocolchicine 0.005 mg/100 g intraperitoneal injection.
destorted star metaphase, which showed a tendency to return to normal with the appearance of telophase at the 12th hour.

An experiment with intraperitoneal injection of thiocolchicine was carried out. Mitoses were at least effectively arrested with 0.01 mg (approximately 1/50 M.L.D.) (Fig. 14.), and scattered diplochromosomes were observed up to 6 hours with this amount. Ana- and telophase showed both signs of recovery at the 6th hour. Accumulation of metaphase was shown in relatively large number with 0.005 mg (approximately 1/100 M.L.D.) (Fig. 15.), while scattered diplochromosomes were seen only in a small number at the 3rd hour. Complete disappearance of ana- and telophase was, however, not observed. With 0.002 mg (approximately 1/250 M.L.D.) (Fig. 16.), abnormal mitoses were hardly seen, of which 1 of 2 cases showed slight accumulation of normal mitoses at the 3rd hour.

4) Thiocolchicine (VII).20)21)

Colchicine-like mitoses appeared an hour after subcutaneous injection of 1 mg of this substance (approximately 1/2 M.L.D.) (Fig. 17.). Abnormal metaphase accumulated in about 20% of the tumor cells after 6 hours, and these changes were mostly taken over by scattered diplochromosomes. 12 hours later both normal and abnormal metaphases took over half of tumor cells, and the increase destorted star metaphase and ball metaphase became remarkable. 24 hours latter, with the appearance of ana- and telophase, the findings of ascites returned to almost normal. Subcutaneous injection of 0.5 mg (approximately 1/4 M.L.D.) hardly showed any effect on the findings of ascites.

5) Condensation product of colchicine-hydrazid with acetone (VIII).20)21)22)

In M.L.D. test (Table 2), 4 of 5 rats survived with subcutaneous injection of 2 mg, while all died with that of 3 mg. However, the rats which survived the
injection of 2mg and 1mg, died in toxic conditions with the second test of 2mg which was administered at 45 days after the first test, while all survived that of 1mg. On the other hand the rat survived by the injection of 2mg at 45 days after

Table 2. The Test for M.L.D. of Condensation Product of Colchicicine-hydrazid with Acetone.

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<th>3rd day</th>
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Indications:
* ...After 45 days from the first test for M.L.D.
** ...3-(p-tolyl sulfonyl) aminotropolone 10mg. subcutaneous injection.
the test for M.L.D. of 3-(p-tolyl-sulfonyl) aminotropolone (XVIII). Judging from the above data, M.L.D. should be decided at 3 mg, but the problem still waits to be solved.

With subcutaneous injection of 2 mg (approximately 2/3 M.L.D.) (Fig. 18.) of this substance, abnormal metaphase was observed at the 1st hour, with only slight decrease of ana- and telophase. At the 3rd hour, destorted star metaphase and necrobiotic metaphase appeared. Scattered diplochromosomes appeared at the 6th hour and was at height after 12 hours. While this scattering remained 24-48 hours, the viscosity of chromosomes increased, resulting in strong tendency of coagulation. Accumulation of metaphase was seen at the height at the 24th hour, and both caryolysis and caryorexis of tumor cells became remarkable at the 48th hour, many giant tumor cells being observed. Ana- and telophase showed tendency to decrease after one hour, and disappeared completely 6 hours later. Normal metaphase did not accumulate throughout the experiment. In this study, 3 of 8 cases died in toxic conditions between 24-48 hours, and 5 other cases showed prolongation of survival. The mean survival of 4, except 1 which died on the 118th day, was 13.7 days, while the mean survival of control was 8.7 days.

With subcutaneous injection of 1 mg (approximately 1/3 M.L.D.) (Fig. 19.), the effect was seen at the height at the 12th hour, followed with recovery at the 24th hour. Scattered diplochromosomes started to appear from the 3rd hour and with the accumulation of abnormal metaphase reached the maximum at the 12th hour, but these changes were hardly seen at the 24th hour. Ana- and telophase which disappeared till 12 hour recovered at the 24th hours. Resting tumor cells showed caryorexis and caryolysis after 24 hours. The survival was, however, not prolonged very much with subcutaneous injection of 1 mg. With subcutaneous injection of 0.5 mg (approximately 1/6 M.L.D.) (Fig. 20), accumulation of metaphase was

Mitotic Cells

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Fig. 19. VIII, b.
Condensation product of colchicine-hydrazid with acetone 1 mg/100 g subcutaneous injection.

Mitotic Cells

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Fig. 20. VIII, c.
Condensation product of colchicine-hydrazid with acetone 0.5 mg/100 g subcutaneous injection.
slightly observed till the 12th hour, and most of abnormal metaphases showed distorted star metaphase. Scattered diplochromosomes were seen slightly at the 6th hour. Ana- and telophase almost disappeared between 3 and 6 hours, and the visible ones were of abnormal type, without ability to complete mitoses. However, normal ana- and telophase recovered completely at the 12th hour.

6) Condensation product of colchicine-hydrazid with methyl ethyl ketone (IX)\textsuperscript{20}
With subcutaneous injection of 5 mg (approximately M.L.D.) (Fig. 21), 2 mg (Fig. 22) and 1 mg of this substance (Fig. 23), slight accumulation of abnormal metaphase

![Fig. 21. IX, a.](image1)
Condensation product of colchicine-hydrazid with methyl ethyl ketone 5 mg/100 g subcutaneous injection.

![Fig. 22. IX, b.](image2)
Condensation product of colchicine-hydrazid with methyl ethyl ketone 2 mg/100 g subcutaneous injection.

![Fig. 23. IX, c.](image3)
Condensation product of colchicine-hydrazid with methyl ethyl ketone 1 mg/100 g subcutaneous injection.

without complete disappearance of polarity, such as star metaphase and necrobiotic star-like metaphase, appeared in small number. Ana- and telophase did not disappear completely with less than 5 mg, but with 5 mg, ana- and telophase only
disappeared between 3-6 hours. With 5 mg, its influence was observed till the 24th hour, while only till the 12th hour with 2 mg or 1 mg.

7) Colchicineamide (X).

Accumulation of abnormal metaphase reached the maximum at the 12th hour after subcutaneous injection of 1 mg of this substance (approximately 1/2 M.L.D.) (Fig. 24), and scattered diplochromosomes were observed remarkably at this time. However, these changes could be hardly observed at the 24th hour and cell division returned to normal. The extracellular outflow of cytoplasm was observed 1-3 hours after injection remarkably. With subcutaneous injection of 0.5 mg (approximately 1/4 M.L.D.) (Fig. 25), scattered diplochromosomes which appeared at the 1st hour did not accumulate very much through the entire course. At the 12th hour star metaphase and necrobiotic star-like metaphase were remarkably accumulated. Ana- and telophase which disappeared up to 6 hours after injection began to appear at the 12th hour, although all of them showed abnormality. At the 24th hour the whole picture returned to normal. With subcutaneous injection of 0.2 mg (approximately 1/10 M.L.D.) (Fig. 26), a slight accumulation of metaphases, which were chiefly of star metaphases, and a formation of abnormal ana- and telophase were observed till the 6th hour. The whole picture, however, showed complete recovery to normal at the 12th hour. The extracellular outflow of cytoplasm was remarkably seen one hour after injection of 0.5 mg and 0.2 mg. A slight accumulation of metaphase, which was mostly star metaphase, was observed up to 6 hours after subcutaneous injection of 0.1 mg (approximately 1/20 M.L.D.) (Fig. 27), while ana- and telophase appeared mingled with a few polypolar formations at the 6th hour. 12 hours later the whole picture recovered to normal.
Few changes were observed one hour after subcutaneous injection of 0.05 mg (approximately 1/40 M.L.D.).

8) Condensation product of colchicine with guanidine (XI).\footnote{20} 3 hours after subcutaneous injection of 10 mg of this substance, a slight degree of accumulation of normal metaphase and also star metaphase was seen. With subcutaneous injection of 20 mg (Fig. 28), there appeared accumulation of normal metaphase with slight increase of star metaphase and a few scattered diplochromosomes. With subcutaneous injection of 30 mg and 40 mg, slight changes were seen as with 20 mg, while both of them died 24-48 hours after injection.
Add. 1. S-ethyl thiocolchicine (XII). Due to the shortage of the material, subcutaneous injection above 2mg could not be studied. The result observed with subcutaneous injection of 2mg. and lesser dosage did not show any influence.

Add. 2. 5-Amino-4 (β-hydroxy-7-phenyl-propyl) tropolone triacetate (XIII). Effect of arresting mitoses was not observed with this substance. It has, however, interesting muscle relaxing effect.

Hindleg paralysis occurred 10 minutes after 30mg was injected into the abdominal cavity of healthy rats, and complete paraplegia was observed 15 minutes later. Paralysis of the forelegs recovered at 45 minutes, with remaining paralysis of the hind-legs which recovered an hour later. The rats ran around again. No respiratory paralysis was observed. 2 of 4 died on the 2nd and 4th day. With intraperitoneal injection of 40mg paralysis of both legs occurred 10 minutes later, and complete loss of movement was observed between 15 and 30 minutes. The paralysis of the forelegs recovered an hour later, while the hind-legs returned to normal an hour and half, or 2 hours later. Respiratory paralysis was not seen. 2 cases which were used for the test died on the 2nd and 4th day, respectively. The above mentioned state was hardly seen in cases of intraperitoneal administration of 5-amino-4 (β-hydroxy-7-phenyl-propyl) tropolone (XIV).

It is interesting to notice that this fact coincides with the reports that colchicine is a poison to nerve.

DISCUSSION

1) In evaluating the effect on mitotic activity of colchicine derivatives morphological changes were chiefly discussed with little consideration on the duration of effect. However, it is doubtful whether a short-acting substance produces the suppressive effect on division of tumor cells or not.

Unquestionably, the strength of action depends on the time factor. If the minimum dose to induce accumulation of abnormal metaphase and disappearance of anaphase and telophase, without consideration on the duration of effect, is decided as minimum effective dose, the following 6 substances have effective dose lesser than M.L.D., i.e., colchicine, T.M.C.A. methyl ether d-tartrate, thiocolchicine, thiocolchicine, condensation product of colchicine-hydrazid with acetone and colchicineamide as reported in this paper. The M.L.D./M.E.D. is 2, 100, 10, 2, 6 and 20, respectively. If the mimimum dose which induces abnormal metaphase accumulation and anaphase and telophase disappearance up to 12 hours is decided as M.E.D. 12 hours, the M.L.D./M.E.D. 12 hours of the above 6 substances are 2, 10, 5, 2, 3 and 4, respectively (Table 3). If the above effect is considered up to 24 hours, there are 3 substances, i.e., T.M.C.A. methyl ether d-tartrate, thiocolchicine and condensation product of colchicine-hydrazid with acetone, and their M.L.D./
M.E.D. 24 hours are 2.5, 5 and 1.5, respectively.

2) From the above experiment, when the side chain of B and C ring of colchicine are studied in details, in B ring, -NH₂ is superior to -NHCOCH₃ in strength, and in C ring, the strength follows -SCH₃, -NH-N=CH₂CH₃, -OCH₃ and -SH in the order given.

Therefore, colchicine derivatives with the following structure seem to have an interesting effect.

![Chemical structures]

**Table 3.**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colchicine</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T.M.C.A. methyl ether d-tartrate</td>
<td>50</td>
<td>25</td>
<td>10</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td>Thiocolchicine</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Thiocolchicine</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Condensation product of colchicine-hydrazid with acetone</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Condensation product of colchicine-hydrazid with methyl ethyl ketone</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Colchicine amide</td>
<td>20</td>
<td>10</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

M.E.D. 24 hours are 2.5, 5 and 1.5, respectively.

**Experiment (2)**

Repeated Administration with Colchicine Derivatives

Prolongation of survival period, and cytological changes were studied by repeated administration of colchicine derivatives. The amount of administration was selected enough to suppress mitoses. Side-effects during administration was controlled fairly well by subcutaneous injection of 2 cc of physiologic saline solution per 100 g body weight a day. The body weight of rat was average 100-120 g, with exceptional use of 120-150 g.

1) Solitary Continuous Administration of Trimethylcolchicinic Acid Methyl Ether d-tartrate.

With continuous administration of this substance, first injection of 0.5 mg followed.
with 0.2 mg every 12 hours was found to be the most effective method. When the total dosage by continuous administration was over 2 mg per 100 g body weight, many died in toxic conditions. With continuous administration 4 days after transplantation, accumulated metaphase, especially, the abnormal metaphase reached its maximum 48 hours after the beginning of injection, when total number of metaphase occupied about 40% of tumor cells and most part of them showed abnormal findings. Scattered diplochromosomes reached its maximum 24 hours after injection, and at the 48th hour star metaphase, ball metaphase and necrobiotic metaphase were chiefly found. The accumulated metaphase remarkably decreased 60 hours after injection, accompanied with conspicuous decrease of numbers of tumor cells in 1 mm³. Telophase was, however, observed in a small number at this time. It was impossible to wipe them out by further administration. The destruction of resting cells was observed 12-24 hours in a form of extracellular outflow of cytoplasm. The appearance of giant cells was outstanding from the 4th day. Ana- and telophase returned nearly to normal 4 days after the injection of substance. The survival period was prolonged in comparison with that of control.

2) Solitary Intermittently Repeated Administration of Thiocolchicine.

In cases of continuous administration of this substance, it was found most effective to inject 0.1-0.05 mg every 12 hour. When the dosage of continuous administration reached 0.5 mg in total, many died in toxic conditions. With continuous subcutaneous administration of thiocolchicine at the 4th day after transplantation, the same findings as in the case of T.M.C.A. methyl ether d-tartrate were observed. The accumulation of metaphase to reach its maximum was delayed, which was 60 hours after injection, while scattered diplochromosomes were found at most at the 48th hour. Necrobiotic and necrotic pictures of resting cells became remarkable at the 48th hour, fairly large number of giant cells appeared from the 72nd hour, which was accompanied with active phagocytosis by many inflammatory cells, such as leucocyte, monocyte, etc.. The survival period prolonged as much as with trimethylcolchicinic acid methyl ether d-tartrate, but less infiltration of tumor cells was found at autopsy. In case of intraperitoneal continuous administration at the 4th day after transplantation, the survival period could be prolonged more than by subcutaneous injection. This tendency was found more conspicuous in rats 4-12 hours after transplantation than in those 4 days after transplantation. The method of administration was studied on rats 4 hours after transplantation with the results shown in Fig. 29. The first and second groups survived longer than the control, but they survived lesser than the third group, which had 0.1 mg 4 times for the earlier administration followed with enough supplemental administration. The picture of destruction of cell division
is shown in Fig. 30. However, in every group, considerable decrease of body weight was noticed by administration in the earlier period.

3) Alternating and Combined Use of Colchicine Derivatives.

Four colchicine derivatives, i.e., T.M.C.A. methyl ether d-tartrate, thiocolchicine, colchiceinamide and condensation product of Colchicine-hydrazid with acetone, were injected alternatingly and continuously into the abdominal cavity as shown in Table 4 and Fig. 31, 4-12 hours after transplantation. By such alternating combined method, rats survived about 20 days after transplantation. The process of destruction of cell division is shown in Fig. 32. After starting administration, metaphase gradually started to accu-

Table 4.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dosage (M.L.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimethylcolchincic acid methyl ether d-tartrate 0.5mg/100g</td>
<td>1/10</td>
</tr>
<tr>
<td>Trimethylcolchincic acid methyl ether d-tartrate 0.2mg.</td>
<td>1/25</td>
</tr>
<tr>
<td>Thiocolchicine 0.05mg.</td>
<td>1/10</td>
</tr>
<tr>
<td>Thiocolchicine 0.02mg.</td>
<td>1/25</td>
</tr>
<tr>
<td>Colchiceinamide 0.1mg.</td>
<td>1/20</td>
</tr>
<tr>
<td>Condensation Product of Colchicine-hydrazid with acetone 0.2mg.</td>
<td>1/15</td>
</tr>
</tbody>
</table>
and took over about 40% of tumor cells on the 3rd day, which was accompanied with abnormal metaphase, especially with scattered diplochromosomes. Thereafter, cell division decreased and it looked as if it disappeared. Several normal mitoses appeared at around 20 days. With this alternating and combined administration of colchicine derivatives, the body weight of rats did not decrease during the whole course.

DiscussiOn

The effect of colchicine derivatives to prolong life is still unsatisfactory. Solitary intermittent administration of thiocolchicine and alternating combined administration of 4 colchicine derivatives in a earlier period after transplantation showed demonstrable prolongation of life.

In case of intermittent repeated administration of thiocolchicine, decrease of body weight was seen in the earlier period of administration, while intermittent administration of 4 derivatives did not show decrease of body weight. It may be better to use the latter than the former in case of human application.

Experimental (3)

Clinical Application of Colchicine Derivatives

The picture of tumor cells in two clinical cases with cancerous pleurisy was studied with alternating combined use of colchicine derivatives, which was tested in animal experiments.

Case 1. Diagnosis. Pleuritis carcinomatosa dextra. 56 years old woman. After middle lobe lobectomy was carried out for primary cancer of the right lung which was confined to segment 4 (Plate I, Figs. 2 and 3), many tumor cells were demonstrated in the punctate of right pleural cavity on the 11th postoperative day (Plate, Fig. 4). Mitoses were found in nearly 2% of tumor cells. With intrapleural
injection of 20 mg T.M.C.A. methyl ethyl d-tartrate which was dissolved in 5 cc of water, chromosomes in metaphase made the so-called ski pair and they were in disorder (Pl. II, Fig. 5). Its accumulation was observed with lapse of time. 10 mg of thiocolchicine which was dissolved in 2 cc of propylene glycol was injected into the pleural cavity 24 hours later. Destruction and accumulation of cell division in metaphase were both remarkably shown, which occupied about 20% of tumor cells at the 33rd hour (Fig. 33). The type of destruction of metaphase which appeared during this time was chiefly scattered diplochromosomes (Pl. II, Fig. 6), distorted star metaphase, ball metaphase, coagulation and necrobiotic metaphase. Anaphase and telophase completely disappeared after injection. Though the changes of resting cells were slight, giant cells appeared. After administration of thiocolchicine, migration of leucocytes increased and phagocytosis of tumor cells was remarkable. 48 hours later, tumor cells completely disappeared (Table 5). Slight migration of leucocytes and monocytes together with fibrin was seen.

Table 5. Pleuritis carcinomatosa 56 yrs. female pleural effusion

<table>
<thead>
<tr>
<th>before injection of tumor cells</th>
<th>counted numbers</th>
<th>numbers of normal metaphase</th>
<th>numbers of abnormal metaphase</th>
<th>numbers of normal anaphase and telophase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection after</td>
<td>1000</td>
<td>4</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>3 hrs.</td>
<td>500</td>
<td>1</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>5 hrs.</td>
<td>500</td>
<td>0</td>
<td>46</td>
<td>0</td>
</tr>
<tr>
<td>9 hrs.</td>
<td>250</td>
<td>0</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>24 hrs.</td>
<td>Thiocolchicine 10 mg. intrapleural injection</td>
<td>40</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>27 hrs.</td>
<td>150</td>
<td>0</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>30 hrs.</td>
<td>88</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>48 hrs.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6 days</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7 days</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10 days</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15 days</td>
<td>Pleural effusion is not obtained.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 33.
Case 1. Pleuritis carcinomatosa. (56 years old woman) Incidence of arrested metaphase in tumor cells.
in pleural punctate on the 6th, 7th and 10th day. It became impossible to ob-
tain punctate 15 days after injection. Patient complained of severe chest pain
when pleural injection was performed, followed with transient accumulation of
exudate. Chest pain appeared from the 3rd hour after injection, increased with
the severity of action of drug, and disappeared when it was impossible to remove
punctate. The patient became free of complaint and was discharged from the
hospital about 2 months later. After about one year, she died with recurrence
of the disease.

Case 2. Diagnosis. Malignant struma with metastasis to the left lung com-
plicated with carcinomatous pleurisy. 63 years old man. The x-ray picture (Pl.
II, Fig. 7) showed remarkable accumulation of pleural exudation. Pleural punctate
(1,800 cc) disclosed many tumor cells (Pl. II, Fig. 8). 3 mg thiocolchicine dissolved in
propylene glycol was injected into the pleural cavity and 2 mg dissolved in 1 cc
of propylene glycol which was added to physiologic saline solution was injected
by the intravenous route by drops. 6 mg thiocolchicine was injected 4 times
intravenously by drops twice every other day, followed with 4 mg colchicine 2
days later by the same method of administration. However, severe diarrhoea
continued for 10 days, and the injection was suspended temporarily. Later on 10
mg T.M.C.A. methyl ether d-tartrate followed with 20 mg of the substance 6
days later was administered. The following day 10 mg thiocolchicine was given
and 2 days later, followed with 10 mg colchiceinamide and 2 days later, followed
with 10 mg condensation product of colchicine-hydrazid with acetone which
caused leucopenia. Consequently, the administration had to be suspended tem-
porarily. With such method of administration, tumor cells decreased and were
replaced with fibrin formation (Pl. II, Fig. 9). With these changes of punctate, the
x-ray shadows of lung diminished and the pleural punctate could not be removed
(Pl. II, Fig. 10). Preexisting chest pain, cough and dyspnea were greatly ameliorated.
Leucopenia recovered within 10 days. Treatment was resumed one month later by
administering 80 mg T.M.C.A. methyl ether d-tartrate, 27 mg thiocolchicine, 32 mg
colchiceinamide and 10 mg condensation product of colchicine-hydrazid with
acetone. However, cancer cells resistant to drug gradually appeared with recur-
rence of symptoms and the patient died 6 months later. At autopsy, pleura was
remarkably hypertrophied, and 500 cc pleural exudate accumulated. Tumors on
bilateral neck were not greatly influenced.

DISCUSSION

According to Lits25), the first application of colchicine on human cancer was
made by Dominici26) followed with reports by Oughterson27), von Brücke28),
Seed29), and others. The effect, though favorable, was far from being satisfac-
tord. Reports of colchicine derivatives on human neoplasm were published by Moeschlin, Bock, Storti and Akaishi, the former investigators using demecolcine.

According to the data by Moeschlin, demecolcine can well be recommended for continual oral therapy of chronic myeloic leukemia.

Of 2 clinical cases herein reported, case 1 was early postoperative occurrence of carcinomatous pleurisy, in which mitoses occupied 2% of tumor cells. It showed mitoses in active stage. Remarkable destruction of cell division was observed by direct intrapleural injection. Case 2 was in a rather late stage of the disease with few mitoses of tumor cells. Therefore, it took time to destroy tumor cells with little influence on pulmonary metastasis. The pleural effusion was, however, transiently held down. From the above data, in case of applying colchicine derivatives, it seems that the more tumor cells in wandering and active mitoses the more the destruction of tumor cells. It is, however, impossible to expect complete chemotherapy of human cancer with present colchicine derivatives.

SUMMARY AND CONCLUSION

1) Study was made on suppressive effect on cell division with colchicine and tropolone derivatives, of which four i.e., T.M.C.A. methyl ether d-tartrate, thiocolchicine, colchicineamide and condensation product of colchicine-hydrazid with acetone had stronger effect than colchicine. These involved different time factors to show their effect. In order to decide the degree of strength, it was found that study on M.L.D./M.E.D. was not enough, but necessary to compare M.L.D./M.E.D. 12 hours with M.L.D./M.E.D. 24 hours. In conclusion, it was suggested that in the derivatives of colchicine the sulfur molecule and –NH–N =C<CH₃CH₃ on the C–ring of colchicine play a comparatively important role.

2) With colchicine derivatives it seems favorable with less toxicity, to use combined method of administration than its solitary use to destroy tumor cells of rats transplanted with Yoshida's sarcoma.

3) Cytological changes were observed in studying the application of colchicine derivatives on 2 clinical cases. When tumor cells were in wandering and had many mitoses, the division of tumor cells were suspended and destroyed in metaphase. However, it is impossible to expect complete chemotherapy of human cancer with present colchicine derivatives. Further investigation are needed on new derivatives and chemical agents which destroy resting tumor cells and on the use of combined administration.
REFERENCES

23) Nozoe, T., Kitahara, Y., and Doi, K.: to be published.
要  旨
不飽和7員環状化合物、特に Colchicine 誘導体の制癌作用に関する研究

赤 石 健一
(東北大学医学部附属外科教室)

Colchicine 誘導体を主とした20種の不飽和7員環状化合物を吉田内腫に試みた。Screening test で colchicine より効果のあったのは T. M. C. A. methyl ether d-tartrate, thiocolchicine, colchiceinamide 及び condensation product of colchicine-hydraizid with acetone の4種であった。これらはそれぞれ作用の持続時間が異なるので、これを詳細に検討した。作用強度を表わすためには M.L.D. M.E.D. のみでは不充分であり, M.L.D. M.E.D. 12 hrs. 及び M.L.D. M.E.D. 24 hrs. を比較することが必要であった。この結果は colchicine のC-環では破壊原子及び -NH-N=CH=CH₃ 基が比較的重要な意義を持つことを示した。また colchicine 誘導体を連続投与する場合は単独で用いるよりも併用した方が毒性少なくして同じような効果をあげた。これを臨床的に用いた場合には、腫瘍細胞が亜変状態にあって細胞分裂の盛んなものは破壊され易く、腫瘍を形成したものには余り有効ではなかった。
The findings of rat ascites at the 12th hour after subcutaneous injection of thiocolchicine 0.1 mg.