Pharmacology of Mitomycin C

I. Toxicity and Pathologic Effects*

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SUMMARY

The toxicity of mitomycin C has been studied in mice, rats, cats, dogs, and rhesus monkeys. Fatal poisoning was uniformly protracted in all five species. Its features were anorexia, steady losses in weight, emesis (in cats and dogs), diarrhea, dehydration, and delayed deaths. Fevers were common in dogs.

The agent had notable hematologic effects. In dogs these included reticulocytopenia, leukopenia, and decreased numbers of nucleated cells in bone marrow aspirates. Hypoplasia of bone marrow, lymphoid tissue damage, and lesions in intestinal epithelium were common pathologic effects in dogs, rats, and monkeys. There were additional lesions: widespread hemorrhages in dogs and monkeys, damage of the epithelium of the glandular stomach in rats, necrotizing nephrosis in monkeys, and liver damage in dogs. Intraperitoneal injections had a direct toxic effect on the peritoneum of rats.

These studies suggest that suppression of hematopoiesis and intestinal injury account for the lethal effects of mitomycin C. The close association of lesions in normal, proliferating tissues with antitumor effects is a pharmacologic property shared by most known chemotherapeutic agents.

Mitomycin C, isolated by Wakaki et al. (20), is one of a related series of antibiotic substances which were recently discovered among the products of fermentation of a new species of Streptomyces (6, 17). Though active against a broad spectrum of gram-positive bacteria, particular interest has centered on its chemotherapeutic effectiveness against experimental tumors in rodents. In this respect mitomycin C appears outstanding because of the wide variety of such neoplasms, which it either inhibits or destroys outright (18, 19). Early clinical reports have indicated its activity against malignant lymphoma, chronic leukemia, and some epithelial tumors (15).

The present study was begun to provide pharmacological data for a cooperative clinical trial of mitomycin C in this country (3). Its toxicity and pathologic effects in laboratory mammals are described in this paper. Subsequent reports will be concerned with its physiological disposition and metabolism.

MATERIALS AND METHODS

The rodents used were males of the Hauschka ICR line of Swiss mice, 20–30 gm. (Millerton Farms, Millerton, N.Y.) and of the CFN line of Wistar rats, 200–300 gm. (Carworth Farms, New City, N.Y.). They were fed Purina Laboratory Chow and water ad libitum. Young rhesus monkeys and adult cats and mongrel dogs of both sexes were used only after the animals had been isolated and observed for at least 4 weeks. At the beginning of the isolation period the monkeys were tuberculin-tested; the dogs were passively immunized with homologous, bivalent hepatitis and distemper antiserum and vaccinated with live, chick embryo-modified distemper virus. The dogs

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were also treated with vermifuges for intestinal parasites and dusted with pyrethrum-DDT mixtures. Isolation was terminated only if the animals consumed their daily allotment of food and if they were free of obvious signs of illness such as purulent discharges from eyes and nose, cough, hyperthermia, and diarrhea. The monkeys were fed dried pellets (Purina Monkey Chow) and the cats, milk and canned diet (C/D, Hill Packing Co., Topeka, Kan.); both species had free access to water. The dogs were given water freely and measured amounts of a mixture of ground meat and Purina Dog Chow sufficient to maintain constant weight; their diet was supplemented once or twice weekly with lightly cooked liver. Daily measurements were made of the dogs' intake of food and water, the volume and specific gravity of their urine, rectal temperature, and weight.

Mitomycin C was dissolved in 0.85 per cent NaCl buffered to pH 6.8 with 0.01 M sodium phosphate; 2 mg/ml was the highest concentration conveniently obtained by brief warming (<60°C). The solutions were used within 1 hour. These conditions were adopted to take account of the instability of the agent (20). Mice and rats were given injections intraperitoneally or were intubated intragastrically with doses in the constant volume of 0.01 ml/gm. LD50 values were calculated (8) after a sufficient number of doses were given, decreasing serially by a factor of \( \frac{1}{2} \), to obtain both 100 and 0 per cent mortality. At least ten mice and six rats were used per dose. Oral doses were given to animals that had been starved overnight. All animals were observed for 14 days after treatment.

Blood samples were drawn from the external jugular veins of preprandial, unanesthetized dogs for hematological and biochemical studies (10). The analytical procedures were the same as those described in the earlier work except for the use of undiluted plasma in the determination of the one-stage prothrombin time. In addition, alkaline phosphatase was measured in serum by the standard Bodansky procedure with a modification employed for determining the phosphate released (5). Capillary blood for hematological measurements was obtained from toe pads of unanesthetized monkeys.

Tissues for microscopic study were obtained from rats anesthetized with ether and from dogs and monkeys anesthetized with pentobarbital sodium; the animals were killed by exsanguination. Specimens from rats were fixed with Zenker-formol except for sternum, which was fixed with Vandergrift's reagent. Dog and monkey tissues were prepared with neutral formaldehyde (10 per cent formalin). All sections were stained with hematoxylin-eosin. The tissues studied included specimens of skeletal and cardiac muscle, thyroid, salivary gland, thymus, tonsil, lung, esophagus, lymph nodes, gonad, all abdominal viscera, and bone marrow (in sternum of all three species and in vertebrae of dogs and monkeys).

### TABLE 1

<table>
<thead>
<tr>
<th>Species</th>
<th>No. successive daily doses</th>
<th>LD50 (mg/kg/day)</th>
<th>10/90 confidence limits (mg/kg/day)</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td>1</td>
<td>8.5</td>
<td>7.6-9.7</td>
<td>1.29</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2.5</td>
<td>1.9-2.8</td>
<td>1.25</td>
</tr>
<tr>
<td>Rats</td>
<td>1</td>
<td>2.5</td>
<td>2.2-2.8</td>
<td>1.22</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.0</td>
<td>0.8-1.2</td>
<td>1.21</td>
</tr>
</tbody>
</table>

### MITOMYCIN C: SINGLE INTRAPERITONEAL DOSES

<table>
<thead>
<tr>
<th>DOSE (mg/kg)</th>
<th>MICE</th>
<th>RATS</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>35/35</td>
<td>20/35</td>
</tr>
<tr>
<td>10</td>
<td>26/35</td>
<td>15/25</td>
</tr>
<tr>
<td>5</td>
<td>0/35</td>
<td>5/21</td>
</tr>
<tr>
<td>2.5</td>
<td>10/21</td>
<td>10/21</td>
</tr>
<tr>
<td>1.3</td>
<td>0/21</td>
<td>2/21</td>
</tr>
</tbody>
</table>

**Table 1.** Toxicity of Mitomycin C given intraperitoneally in mice and rats.

**Table 2.** Mitomycin C: single intraperitoneal doses.

**Chart 1.** Fate of mice and rats. Each closed circle represents the death of one individual.

### RESULTS

**Toxic Effects in Mice and Rats**

**Course of intoxication.**—The various intraperitoneal doses used to compute the LD50 values in Table 1 and Chart 1 caused similar effects whether given by single or repeated injection. Fatal intoxication was prolonged and was characterized by weight loss, disheveled appearance, "bloody" masks (in rats), depression, diarrhea, and dehydration. Following single doses near the LD50, losses in weight were evident within 24 hours in rats and within 2 or 3 days in mice; diarrhea and the other signs appeared at 3 or 4 days in both species. Death was conspicuously delayed (Chart 1). Ani-
mals surviving median lethal doses or one-half of such doses showed typical signs of intoxication with maximum intensity between 4 and 8 days after injection. Recovery was rapid and usually complete within the next week.

**Oral doses.**—Since mitomycin C is unstable in mildly acid conditions (20), it was conceivable that administration in a mildly alkaline buffer might increase its oral toxicity by protecting against gastric acidity. As seen in Table 2, bicarbonate increased toxicity in both species; the effect was not statistically significant in mice. In rats the increase was twofold and probably significant ($P < 0.05$ by the method of Litchfield and Wilcoxon [8]). The course of intoxication was similar in animals given solutions either in bicarbonate or water and was like that seen after intraperitoneal doses.

**Pathologic effects in rats.**—The prolonged intoxication and delayed deaths caused by mitomycin C in the animals described above were probably associated with characteristic lesions. These were studied in an additional group of rats given injections intraperitoneally of five successive doses of 1 mg/kg/day (the previously determined LD$_{50}$ by the five-dose schedule, Table 1). From a total of fifteen treated animals groups of three were selected for greatest weight loss and autopsied, respectively, at 4 days (24 hours after the fourth dose), 7 days (72 hours after the fifth dose), and at 9 days. The 4-day animals had lost 13–16 percent of their initial weight; the 7-day animals, 30 percent; the 9-day animals, 30–35 percent. Four of the six autopsied at 4 and 7 days had fluid stools. All six killed at 7 and 9 days were depressed, dehydrated, and untidy, and had "bloody" masks. The remaining animals also lost 18–30 percent of their initial weight by 9–10 days; one died at 14 days, and the other five recovered.

There were several gross abnormalities in the autopsied groups. The three 4-day animals had viscous, cloudy ascites. (Abundant quantities of clear pleural and ascitic fluid had been seen in animals that had died after single intraperitoneal doses of 20 or 10 mg/kg though not in rats given 5 or 2.5 mg/kg.) In the 7- and 9-day rats there were yellow flecks on the surface of the pancreas and opaque, white areas on the visceral peritoneum. The thymuses and spleens were small in all, and the mesenteric lymph nodes were red in eight of the nine. The intestines contained excessive amounts of fluid in four of the six killed at 4 and 7 days, and petechial hemorrhages were present in the mucosa of colon and glandular stomach of the 7- and 9-day rats.

Microscopic lesions were numerous. All nine animals showed a generalized peritonitis that was the probable cause of the ascites. At 4 days the peritoneal reaction consisted mainly of infiltration of inflammatory cells. By 7 and 9 days fat necrosis was present throughout the visceral peritoneum, mesentery, and peripancreatic tissues. The mucosa of the glandular stomach and colon were severely affected. At 4 days the stomach was normal. At 7 days diffuse ectasia, focal atypia, and distortion of glandular architecture were present in the basal portions of the mucosa; by 9 days this damage was still greater and extended to the surface. Similar changes were evident in the colon at 4 days; at 7 days they were more severe, but small areas of epithelial regeneration were apparent; by 9 days the epithelium was entirely normal in one and almost completely repaired in the other two. These alterations corresponded closely in time with the diarrhea described above. The small intestine was abnormal in only the 4-day rats; the epithelial cells showed some swelling and loss of nuclear polarity. The thymuses were involuted, moderately at 4 days and completely at 7 and 9 days. All hematopoietic elements were reduced in amount in all 4- and 7-day and in one 9-day spleen; in the other two 9-day rats only the red pulp was affected. The lymph nodes were normal except for the presence

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**TABLE 2**

**EFFECT OF SODIUM BICARBONATE ON THE ORAL TOXICITY OF MITOMYCIN C IN MICE AND RATS**

Animals were starved overnight and then intubated by mouth with 0.01 ml/gm* of solutions of mitomycin C in either water or 0.1 M NaHCO$_3$. They were observed for 14 days.

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose in:</th>
<th>Mortality</th>
<th>LD$_{50}$ (mg/kg)</th>
<th>19/80 confidence limits (mg/kg)</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water (mg/kg)</td>
<td>0.1 M NaHCO$_3$ (mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mice</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>10</td>
<td>10/10</td>
<td>23</td>
<td>19–29</td>
<td>1.27</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>8/10</td>
<td>17</td>
<td>12–23</td>
<td>1.69</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>8/10</td>
<td>17</td>
<td>12–23</td>
<td>1.69</td>
</tr>
<tr>
<td><strong>Rats</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>40</td>
<td>10</td>
<td>5/6</td>
<td>30</td>
<td>21–42</td>
<td>1.34</td>
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<tr>
<td>20</td>
<td>10</td>
<td>0/6</td>
<td>14</td>
<td>11–19</td>
<td>1.42</td>
</tr>
<tr>
<td>10</td>
<td>5/6</td>
<td>6/6</td>
<td>14</td>
<td>11–19</td>
<td>1.42</td>
</tr>
</tbody>
</table>

* The 40-mg/kg doses were given in the volume of 0.02 ml/gm.
of erythrocytes in the sinuses in one 4-day and one 7-day rat. In sternal bone marrow the nucleated hematopoietic cells were reduced in quantity; the reductions were estimated to be 10 per cent at 4 days, 15–40 per cent at 7 days, and 10–50 per cent at 9 days (Fig. 1). These depletions were not impressive when compared with the effects of other cancer chemotherapeutic agents such as 1,4-dimethanesulfonoxybutane, Myleran (4), or thioguanine (10). The testes were unaffected except in one 7-day rat in which there was spermatogenic arrest. Edema and chronic inflammation were seen in the salivary glands of two 9-day animals. Focal myocarditis was also present in two of the 9-day rats.

**Toxicity in Cats**

A small number of adult cats of both sexes received large, single doses intravenously to test for precipitant, acute effects. A pair given 10 mg/kg (approximately 4–8 times the LD₅₀) showed no changes until 2 hours, when they began to salivate and vomit. During the first 8 hours there were no postural or locomotory disturbances, and respiratory and cardiac rates were unaltered. At 24 hours and later the salivation and vomiting continued, and the animals stopped eating, lost weight, and appeared depressed. During the 3d day one of the pair had a 1-minute episode of generalized, clonic spasms, followed by extreme depression and a moribund state. The animal was dead before the end of this day; the other died at 4 days.

In two pairs given, respectively, 5 and 2.5 mg/kg intoxication was longer, and the following sequence of disturbances appeared: anorexia and emesis, weight loss, depression, ataxia of hind quarters, and diarrhea. The last became evident about 24 hours before death. All four died between 5 and 7 days. In pairs given 1.25 or 0.63 mg/kg the only obvious change was weight loss which became maximal between 7 and 11 days and from which the animals recovered.

**Toxicity in Dogs**

*Toxicity.*—The data in Table 3 show that the toxicity of multiple intravenous doses was nearly identical with that caused by single doses: the approximate LD₅₀ of single doses was 1 mg/kg, while that found by giving ten successive daily doses was, in total, 1.25 mg/kg. Animals given single lethal doses survived about as long as individuals treated with multiple doses. Table 3 also shows that the agent was about equally potent by the two parenteral routes but only about one-eighth as active orally. The behavior of poisoned animals (see below) was similar regardless of the route of administration, and there was no evidence for specific, local damage, e.g., neither venous thrombosis at intravenous injection sites, nor tenderness, swelling and warmth at intramuscular sites, nor excessive emesis that might suggest direct damage to the lining of the stomach.

**Table 3**

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Dose (mg/kg/day)</th>
<th>No. of successive daily doses</th>
<th>Mortality</th>
<th>Day of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous</td>
<td>2</td>
<td>1</td>
<td>4/4</td>
<td>5, 6, 7, 7S</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>4/6</td>
<td>5, 6, 12, 8</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>1</td>
<td>0/4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intravenous</td>
<td>1</td>
<td>10</td>
<td>1/1</td>
<td>8</td>
</tr>
<tr>
<td>0.5</td>
<td>10</td>
<td>3/3</td>
<td>8, 9, 9</td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>10</td>
<td>2/2</td>
<td>7, 11</td>
<td></td>
</tr>
<tr>
<td>0.125</td>
<td>10</td>
<td>1/2</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>0.065</td>
<td>10</td>
<td>0/2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral</td>
<td>1</td>
<td>10</td>
<td>2/2</td>
<td>9, 13S</td>
</tr>
<tr>
<td>0.5</td>
<td>10</td>
<td>0/2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>10</td>
<td>0/2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intramuscular</td>
<td>0.4</td>
<td>10</td>
<td>2/2</td>
<td>10, 13</td>
</tr>
<tr>
<td>0.2</td>
<td>10</td>
<td>1/2</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>10</td>
<td>1/2</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

* Equal numbers of both sexes used for each dose tested.
† A maximum of ten doses was given on successive days except weekends, i.e., ten doses in 11–13 days.
$\ddagger$ killed for histopathology.

**Table 4**

<table>
<thead>
<tr>
<th>NaHCO₃ (mg/kg)</th>
<th>Mitomycin C (mg/kg)</th>
<th>Mortality</th>
<th>Day of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4</td>
<td>0/2</td>
<td>7, 9</td>
</tr>
<tr>
<td>50</td>
<td>4</td>
<td>0/2</td>
<td>7</td>
</tr>
<tr>
<td>50</td>
<td>2</td>
<td>1/2</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>1</td>
<td>0/2</td>
<td></td>
</tr>
</tbody>
</table>

The possibility that gastric acidity might account for the lower potency by the oral route was tested by giving NaHCO₃ along with single doses of the agent (Table 4). The two controls given only mitomycin in the large dose of 4 mg/kg were little affected: one showed no change; the other vomited...
during the first 24 hours and again during the 7th day. It lost about 5 per cent of its weight by the 7th day and then recovered rapidly. By contrast, all the animals given bicarbonate and mitomycin became severely intoxicated; three died, and the three survivors showed transient episodes of fever at 6 and 7 days (see below). A comparison of these data with Table 4 shows that bicarbonate increased the toxicity of oral doses to about one-half of that found after intravenous injection.

Course of intoxication.—The overt signs of poisoning in dogs were delayed in onset and included all those changes which have been described in mice, rats, and cats. Most of the animals listed in Table 3 were normal for the first 3 or 4 days. Thereafter, they showed anorexia, weight loss, diarrhea, emesis, and fever. The amount of fluid produced by vomiting was not abundant, since it did not significantly reduce the specific gravity or increase the volume of the daily fluid output. The diarrhea was also minimal by contrast with the copious amounts of watery stool caused by such antitumor agents as bis(2-chloroethyl)methylamine (9) or 4-amino-N10-methylpteroylglutamic acid (11). It was, nevertheless, an ominous sign since it was observed in fourteen of the 21 fatalities, but in only six of the nineteen survivors (see Table 3). In five instances blood was present in the diarrheal fluid. Rectal temperatures greater than 103° F. also occurred frequently, for example, in fourteen of the sixteen given the different single intravenous doses listed in Table 3 and in all seven of those receiving repeated intravenous doses between 0.5 and 0.125 mg/kg/day. Most of the fevers began between the 4th and 7th day of intoxication.

As mentioned above, there were no consistent, significant changes in the volume or specific gravity of the daily urine collections. Presumably there were no major functional or morphological disturbances in the renal tubular epithelium.

Hematologic effects.—These were first seen in blood leukocyte counts of three dogs which were part of the toxicity studies described above. The counts, done at 7, 8, and 15 days, respectively, when each of the animals had fever, revealed neutropenia, i.e., 140, <350, and 1050 cells per cu. mm. This abnormality as well as other hematologic defects was seen again in the series of four dogs described in Table 5. The table shows that depression of the neutrophil count occurred in all four animals. In the two receiving 0.2 mg/kg/day and in another pair given 0.1 mg/kg/day (not reported in Table 5) significant numbers of unidentified, blastlike cells (up to 500/cu mm) appeared in peripheral blood by the end of the 1st week of treatment. At this time the neutrophil series consisted largely of immature, unsegmented or "old" hypersegmented cells but relatively few intermediate stages. Table 5 shows other signs of bone marrow injury such as reticulocytopenia, decreased numbers of nucleated cells in bone marrow aspirated from the iliac crest, and decreases in the hematocrit. The last change was particularly significant in dogs 133 and 139, since this pair may have been dehydrated (see below). In view of the bone marrow effects it is noteworthy that thrombocytopenia did not occur. There were no significant changes in blood-clotting time (not reported in Table 5).

It is interesting to compare the occurrence of fever in the four dogs of Table 5 with changes in neutrophil counts. In three there was a close relation between severe neutropenia and hyperthermia; but in 139 fever occurred when substantial numbers of neutrophils were still present in blood. The relation between agranulocytosis and the fevers caused by mitomycin will be discussed below.

Blood biochemical changes.—Each blood sample in Table 5 was analyzed for serum nonprotein nitrogen, blood glucose, and one-stage prothrombin time in plasma. Since no significant changes occurred in these measurements, even in samples drawn when the animals were severely intoxicated, they were omitted from the table. Serum chloride values also remained normal except for small decreases in dogs 133 and 139 on the day of death: the initial chloride values had been 112 and 113 meq/liter, respectively; the final values were 102 and 107. This change was presumably associated with dehydration, since it was seen along with anorexia, diarrhea, and weight loss. The consistent increases in serum alkaline phosphatase shown in Table 5 reflected hepatotoxic effects to be described below.

Pathologic effects.—Five severely intoxicated dogs were autopsied, including dogs 133 and 139 of Table 5 and three animals from the toxicity series of Table 3. One of these three had died 3 days after a single dose of 1 mg/kg; the other two were killed at the times shown in Table 3. Hemorrhages were the most conspicuous gross finding. In all dogs these were petechial in the colon, a finding that undoubtedly accounts for the bloody feces mentioned above. Except for 139, in which the colon alone was involved, hemorrhages were also present in a number of other tissues: muscle of the tongue, pericardium, endocardium, mucosa of trachea and bronchi, serosa of the intestines, anterior mediastinum, adrenal medulla and cortex, renal cortex, and mucosa of esophagus and terminal ileum. Massive
hemorrhages were present in the parietal pleura of the two dogs given the single lethal doses. Other lesions noted in two of the animals were tonsillitis and ulcerations of the buccal or lingual mucosa.

Microscopic examination confirmed the widespread hemorrhages. These were present in the mucosa of the colon in each animal; in two of the five all layers were hemorrhagic. In addition to the sites mentioned above hemorrhages were seen in buccal, lingual, and pharyngeal mucosa, tonsil, lung, heart, and gall bladder. The mucosa of both colon and small intestine showed epithelial atypia and ectasia, but these were not seen in all animals, and they were much less severe than in the rats described above. They were present in the colon of only one dog and in the small bowel of three dogs. In addition, the small intestine of the animal which died at 3 days showed severe karyorrhexis in the basal third of the epithelial glands. There were no changes in gastric mucosa in contrast with the findings in rats. Superficial erosions or ulcerations with bacterial colonies were seen in tonsils, buccal mucosa, and pharynx. There was karyorrhexis and depletion of lymphocytic cells in the lymph nodes and spleen of the dog dead at 3 days; in the dog killed at 7 days after 2 mg/kg intravenously the periphery of the follicles contained decreased numbers of small lymphocytes. Lymphatic atrophy was not evident in the remaining three. The red pulp of the spleen was uniformly congested and contained decreased numbers of nucleated cells; erythrocytes were prominent in the sinuses of lymph nodes. Bone marrow sections were obtained from dogs 133 and 139 and the one killed at 7 days after 2 mg/kg intravenously. In two the marrow was almost entirely devoid of hematopoietic cells. In the third, dog 139, the depletion was less extensive, and the elements remaining were for the most part mononucleated and plasma cells. The liver of dog 139 had moderate lobular

**TABLE 5**

**Weight, Temperature, Blood Cells, and Serum Alkaline Phosphatase in Dogs Given Mitomycin C Intravenously**

Daily doses injected on successive days except during weekends.

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Dose×no. injections (mg/kg/day)</th>
<th>Day</th>
<th>Weight (kg.)</th>
<th>Temperature (°F.)</th>
<th>Hematocrit (ml/100 ml)</th>
<th>Reticulocytes (%)</th>
<th>Neutrophils (10⁹/cu mm)</th>
<th>Lymphocytes (10⁹/cu mm)</th>
<th>Platelets (10⁹/cu mm)</th>
<th>Bone marrow nucleated cells (10⁹/cu mm)</th>
<th>Serum alkaline phosphatase (mg P/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>140</td>
<td>0.4×7</td>
<td>0</td>
<td>15.85</td>
<td>102</td>
<td>46</td>
<td>0.3</td>
<td>16.40</td>
<td>3.40</td>
<td>300</td>
<td>640</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>14.72</td>
<td>101.7</td>
<td>44</td>
<td>0.1</td>
<td>3.02</td>
<td>3.28</td>
<td>280</td>
<td>60</td>
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<tr>
<td></td>
<td></td>
<td>4</td>
<td>13.89</td>
<td>101.6</td>
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<td>0.0</td>
<td>2.99</td>
<td>3.21</td>
<td>250</td>
<td>17</td>
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* Total white blood cell count.
S, killed for histopathology.
DISCUSSION

The effects of mitomycin C are uniform in different mammalian species. Median lethal doses given parenterally in rats, cats, dogs, and monkeys vary within the narrow range of 1.0-2.5 mg/kg. This compares closely with the total dose of 0.75-1.5 mg/kg likely to produce serious signs of toxicity in cancer patients (9). In all five laboratory species lethal doses cause a protracted intoxication that is delayed in onset and characterized by anorexia, steady losses in weight, diarrhea, dehydration, and delayed death. Similar effects have been seen in human beings (3).

Lesions in hematopoietic tissues and the intestinal epithelium are the common pathologic changes in rats, dogs, and monkeys. They probably account for most fatalities in these species as well as the major signs of toxicity in patients (3). They are also the principal toxic effects of most antitumor agents. The close association of such lesions with chemotherapeutic activity is undoubtedly related to the fact that hematopoietic tissues and the intestinal epithelium have the highest rates of proliferation found in the post-fetal mammalian organism (7).

There are several other effects of mitomycin C to be considered. The necrotizing nephrosis seen in monkeys was not found in dogs or rats, and clinical reports have not shown renal damage to occur in human beings (8, 15). The fevers induced by mitomycin in dogs need further study. Most febrile episodes may have been due to secondary infections consequent to hematopoietic inhibition and agranulocytosis. Such associations have been seen in dogs treated with other bone marrow depressants—for example, total-body exposure to ionizing radiations (2) and thioguanine (10). However, in the present animals fever usually appeared before disorganization with foci of single-cell necrosis. Cholestasis and slight fatty change were present in dog 138. These hepatic alterations seemed consistent with the increases in serum alkaline phosphatase noted in Table 3. Finally, the lung of dog 139 showed the changes associated with canine distemper. Severe pulmonary changes were present in only one other dog, i.e., the one that died at 3 days. In this individual the hemorrhages were particularly severe, and they were accompanied by edema fluid and bacterial colonies in alveoli.

TOXICITY IN RHESUS MONKEYS

Pairs of young rhesus monkeys (five females and one male, 3.4-6.1 kg.) received the following series of intravenous doses: 2, 1, and 0.5 mg/kg. The two given the highest dose were killed for histopathological study at 5 and 8 days when death seemed imminent. (The 8-day animal was the one male of the series.) One of the 1-mg/kg animals died at 7 days; the remaining three survived. All animals lost weight during the 1st week, and severe watery diarrhea was present in one of the pair given the 2-mg/kg dose. The three fatally intoxicated animals became depressed several days before death. In the other four monkeys the changes in these formed elements were not impressive. As in dogs, the thrombocyte counts remained within normal limits even in the three given the lethal doses.

Pathologic effects.—Two changes were prominent in the gross appearance of the autopsied pair: hemorrhages and a marked pallor of the renal cortex. Both animals had petechial hemorrhages in the colon; in one hemorrhages were also distributed throughout the same kinds of tissues as described above in dogs. In the other there were massive hemorrhages in the parietal pleura as in the two dogs studied after single, intravenous doses.

The microscopic lesions in both animals included a necrotizing nephrosis (Fig. 2), recent hemorrhages as noted above, and marked atrophy of the mucosa of the colon as well as atypia and swelling of the epithelial cells in the small bowel (Fig. 3). The bone marrows showed only a slight depletion of hematopoietic elements. This seemed due to a deficiency of granulocytes and lymphocytes, since most of the cells present were immature.

FIG. 1.—Section of rat sternum showing moderate depletion of nucleated elements. This represents the maximum marrow damage observed in rats. (Five successive intraperitoneal injections of 1 mg/kg/day. Animal killed at 9 days.)

FIG. 2.—Necrotizing nephrosis in monkey. Portions of the convoluted tubules show necrosis, absence of nuclei, loss of cell boundaries, and protein casts. Some nuclei are irregular and enlarged. (Animal killed 5 days after a single intravenous injection of 2 mg/kg.)

FIG. 3.—Duodenum of the monkey described in Figure 2 showing atrophy and glandular distortion. Epithelial nuclei of glands show irregularities in size, shape, and position. The changes are similar to those observed in rats and dogs.
the end of the first week of intoxication and, in some instances, too early to have been preceded by severe neutropenia (see dog 139, Table 5). With agents like ionizing radiation or thioguanine the onset of fever is more delayed. Another toxic effect—one which was striking grossly in dogs and monkeys—was widespread hemorrhages. These were not associated with thrombocytopenia, elevations of whole blood clotting time, or increases in plasma prothrombin time. Whereas they may have been due to a direct action on vascular endothelium, it is noteworthy that, together with the early febrile responses just discussed, they resemble certain of the properties of bacterial endotoxins.1

Although little is known about the mechanism of action of mitomycin C in mammals, studies with microorganisms point to interference with the de novo synthesis of purines (12) or to the selective inhibition of the biosynthesis of deoxyribonucleic acid (13, 14). Such actions are in accord with the principal effects in mammals which are centered on proliferating tissues. Whatever the proximate biochemical defect may be, it is probably generated soon after injection; since the agent rapidly disappears in vivo and there is no substantial evidence for selective accumulation in any tissue.1

ACKNOWLEDGMENTS

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