PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, **147** 546-550 (1974)

Antimitotic Effects of Dehydroretronecine Pyrrole[1] (38384)

J. R. ALLEN AND I. C. HSU

Department of Pathology, University of Wisconsin Medical School, and Regional Primate Research Center, University of Wisconsin, Madison, Wisconsin 53706

Monocrotaline, a naturally occurring pyrrolizidine alkaloid, has been shown to be responsible for diverse lesions in man and lower animals. Hepatic necrosis (1), cirrhosis (2), neoplasia (3), veno-occlusive disease (4), pulmonary hypertension, and cor pulmonale (5) are the more common pathological alterations attributed to this alkaloid. Monocrotaline pyrrole, a synthetic metabolite, produces lung, heart, and liver lesions similar to those caused by the parent alkaloid (6). However, due to its instability, this metabolite must be administered into the vasculature entering the liver or lungs if the desired injurious effects are to be obtained. Recently in this laboratory a toxic metabolite which has been identified as dehydroretronecine was found in the urine, liver, and blood of rats receiving monocrotaline (7). In the presently reported experiment the morphological changes that develop in dehydroretronecine-intoxicated rats are delineated. It was determined that the major tissue modifications that occur in the affected animals result from the inhibitory effects of this metabolite on rapidly dividing cells.

Materials and Methods. Dehydroretronecine was prepared according to the method of Culvenor (8) and dissolved in 0.1 *M* phosphate buffer immediately prior to use. Sixty-six male, 26-day-old Sprague–Dawley rats weighing 60–70 g were divided into four groups. Three groups consisting of 18 animals per group were given subcutaneous injections of dehydroretronecine at doses of 90, 150, and 250 mg per kg body weight. The 12 rats of the fourth group received a similar volume of phosphate buffer (pH 7). Three rats from each treated group and two control rats were sacrificed at 24, 48, and 72 hr and 1 and 2 wk after injection. When the animals were sacrificed blood was collected for serum electrophoresis and hematological evaluations. Body and organ weights were obtained, and small segments of the tissues were fixed in 10% buffered formalin. Paraffin-embedded tissue sections were cut at 5 μm and stained with hematoxylin and eosin for histological evaluation.

Results. The dehydroretronecine-treated rats failed to gain weight, developed diarrhea, and started to die within 60 hr. After 1 wk all of the animals had either been sacrificed or had died with the exception of five rats from the group receiving 90 mg and one rat given 150 mg. Hematological changes in the experimental animals included a decrease in the white blood cell count, serum albumin, and hemoglobin, and an increase in serum globulins and serum protein at 72 hr and 1 wk (Table I). At necropsy, all of the animals that died or were sacrificed within 72 hr had markedly distended stomachs. Widespread hemorrhage and ulceration of the gastric mucosa also was present in these animals. In addition to the pale bone marrow, there was thymic regression and splenic hypoplasia. However, in those animals that survived beyond 7 days, the above-mentioned tissue changes were less obvious.

In the animals that died or were sacrificed during the initial 72 hr, the major morphological changes were in the stomach, small intestine, spleen, thymus, testes, and bone marrow. Similar tissue alterations were recorded in all animals regardless of the dose of dehydroretronecine. However, these changes were less severe and the recovery more rapid in the animals given the lowest dose of dehydroretronecine. Within 24 hr

[1] The critical review of this manuscript by Dr. J. J. Lalich is gratefully acknowledged. This investigation was supported in part by U.S. Public Health Service Grants HL-10941 and RR-00167 from the National Institutes of Health. Primate Center publication no. 14-003.

TABLE I. Hematological Changes in Rats Given 250 mg Dehydroretronecine.

Analysis	3 Days after treatment		7 days after treatment	
	Control	Experimental	Control	Experimental
WBC ($x10^3/mm^3$)	10.4 ± 1.8	5.9 ± 1.1	7.9 ± 0.9	4.6 ± 1.3
Total protein (g/100 ml)	4.5 ± 0.3	5.0 ± 0.6	4.6 ± 0.1	5.3 ± 0.9
Albumin (%)	59.8 ± 1.8	43.4 ± 7.0	59.0 ± 0.4	37.8 ± 8.0
Globulin (%)	40.1 ± 1.3	56.0 ± 6.1	40.9 ± 0.5	61.2 ± 9.0
A/G	1.48	0.55	1.45	0.59

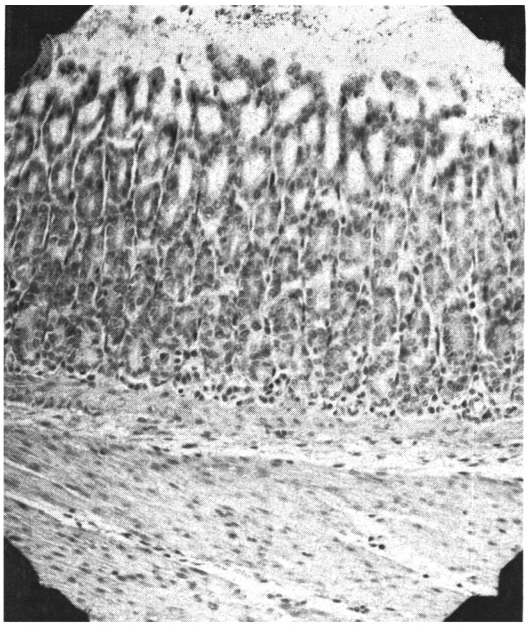
the entire stomach mucosa, muscularis mucosae, and submucosa were affected. The mucosal glands were dilated, the epithelium flattened, and the lumina filled with eosinophilic debris, leukocytes, and cell fragments (Figs. 1 and 2). The limited number of cells that were undergoing mitosis assumed many bizarre configurations, the most common of which was an anaphase lag. There were numerous leukocytes and extravascular erythrocytes in the lamina propria, muscularis mucosae, and edematous submucosa. Congested capillaries in the lamina propria were prominent. Within 72 hr a large percentage of the glands were dilated and mucosal atrophy was obvious due to the decrease in number of epithelial cells. The edema, congestion, and leukocytic infiltrate persisted in the basal mucosa and underlying tissues. The sparsity of mitoses in epithelial cells was strikingly obvious. By day 7 after treatment the reduced thickness of the mucosa still remained apparent. However, the number of dilated glands was reduced, while the edema and inflammatory cells were also less obvious (Fig. 3). By day 14, the gastric mucosa in the surviving animals still remained atrophic; however, the epithelium that was present appeared normal.

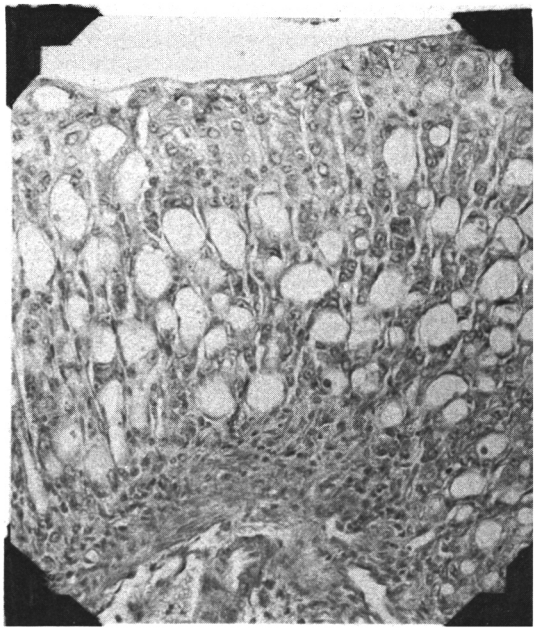
The ulcerated areas that developed primarily at the junction of the squamous and glandular portions of the stomach were associated with necrosis of the muscularis mucosae and submucosa (Fig. 4). Thrombi were prevalent in the vessels underlying these necrotic areas. Only minimal numbers of leukocytes were seen in the base of these ulcers without any evidence of fibrous repair.

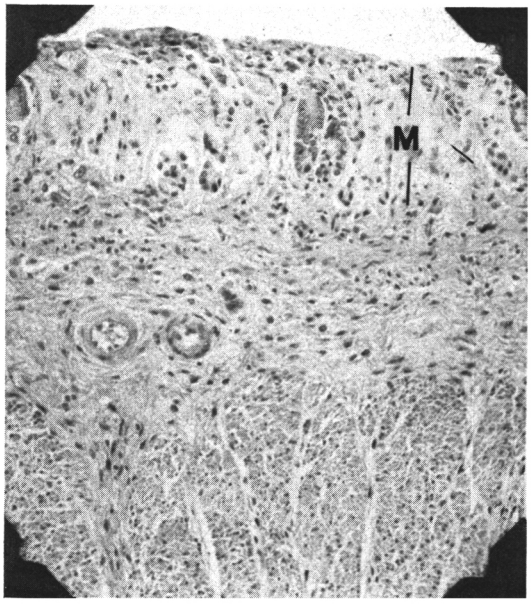
Similar mucosal changes but less severe ulcerations of the small and large intestine were found in the animals given dehydroretronecine. However, by day 7, the mucosal surface of the intestinal tract had reassumed almost normal morphological features.

Within 24 hr there was an obvious decrease in the cortical cell population of the thymus, reduced size of the splenic malpighian corpuscles, retarded spermatogenesis in the tubules of the testes, and a paucity of erythroid, myeloid, and megakaryocytic cells in the bone marrow (Fig. 5). By 48 hr, only a narrow band of cells in the thymus and a few lymphocytes in the spleen were seen (Fig. 6). The cell population of the bone marrow consisted primarily of mature erythrocytes. While these tissue changes persisted for 72 hr, almost complete recovery was seen after 7 days. The number of lymphoid cells in the thymus and spleen had increased, the bone marrow had attained a near normal complement of cells, and active spermatogenesis was present in the seminiferous tubules.

Discussion. The data presented in this report indicate that there are distinct differences in the tissue response of rats to monocrotaline and its metabolite dehydroretronecine. The major tissue changes produced by monocrotaline involve the lungs, heart, and liver. In the lungs, occlusion of many capillaries and small muscular arteries produces pulmonary hypertension (9). This increase in pulmonary arterial pressure is in turn responsible for the development of cor pulmonale. The centrilobular necrosis of the liver is followed by fibrosis and megalocytosis (10). Tumors involving the liver also occur in animals that survive for prolonged periods (2). However, when dehydroretronecine was given to rats either in large doses that produced acute lesions or at lesser concentrations, the chronic forms of pulmonary vascular obstruction and cor pulmonale were not observed. In addition, the acute hepatotoxic effects produced by monocrotaline are not seen when the animals are exposed to







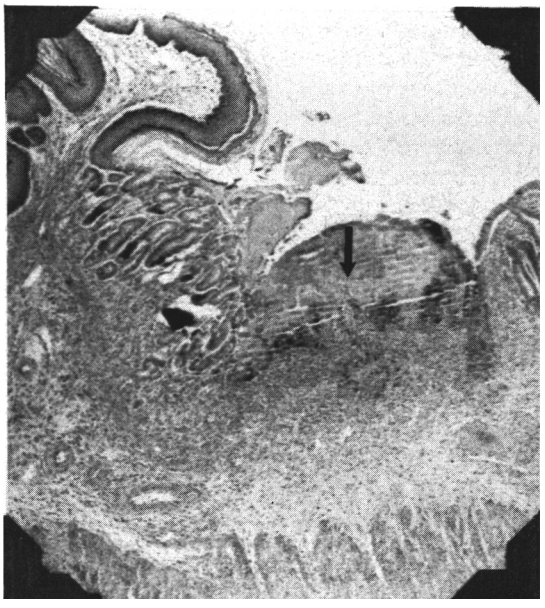


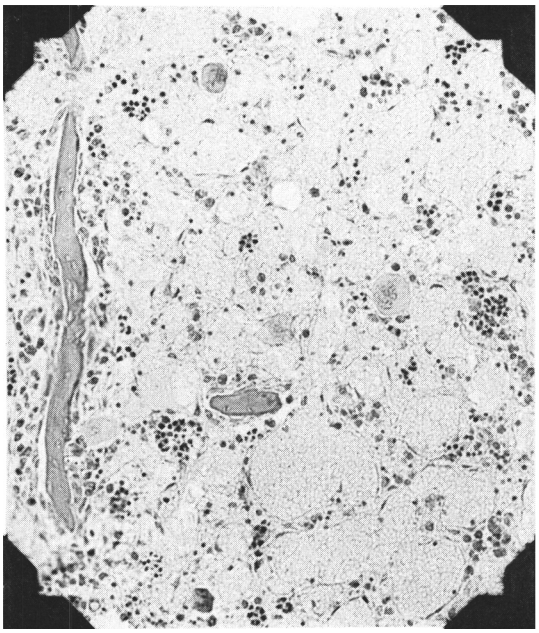
FIG. 1. The stomach wall of a control rat. Hematoxylin and eosin stain; × 55. FIG. 2. Cystic dilatation of the glandular elements and gastric mucosal atrophy are apparent when one takes the magnification into consideration. These cellular alterations were produced by dehydroretronecine within 24 hr. Hematoxylin and eosin stain; × 110. FIG. 3. After 7 days the gastric mucosal atrophy is still evident (M) in rats that were given dehydroretronecine. The mucosal atrophy is believed to be due to the inhibiting influence of the drug on mitosis. Hematoxylin and eosin stain; × 55. FIG. 4. Necrosis of the gastric mucosa (→) near the junction of the glandular and squamous portions

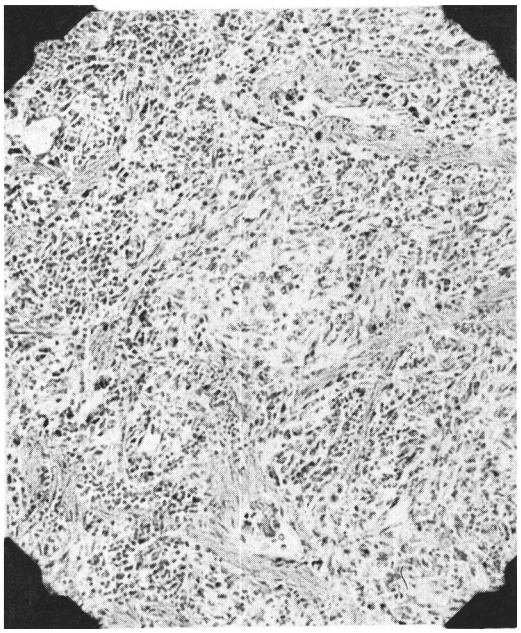
dehydroretronecine. In contrast, these animals develop lesions that are primarily associated with the inhibiting effects that dehydroretronecine has on mitosis. The rapidly dividing cells in such organs as the gastrointestinal tract, bone marrow, thymus, and testes undergo rapid degeneration primarily due to their reduced ability to undergo mitosis. It has also been reported (7) that the prior administration of dehydroretronecine will induce a 30-fold suppression of mitotic activity in partially hepatectomized rats.

This widespread depletion of rapidly dividing cells in multiple organ systems has not been reported to occur in animals given monocrotaline. However, denuded mucosal epithelium and hemorrhage in rats chronically exposed to monocrotaline have been observed (10). While monocrotaline has been reported to inhibit mitosis of hepatic cells (11), such animals were apparently devoid of suppression of cellular multiplication in the spleen, testes, and bone marrow.

In recent experiments (12) tissue distribution of ^{3}H dehydroretronecine was determined. Localization of radioactive material in the glandular portion of the stomach was found to be at least five times greater than in any other tissue. Other organs showing high radioactivity were the liver, kidney, testes, spleen, thymus, and brain. It may be of interest that the radioactivity of the gastric mucosa and the area of dehydroretronecine injection persisted at high levels for a period in excess of 2 wk, while levels in the other tissues declined rapidly. This previously reported study correlated well with the pathological changes that are the subject of this report because the organs with the greatest radioactivity were most severely affected. It is worthy of note that neoplastic changes of epithelium fibroblasts also have been recorded at the site of dehydroretronecine injection many months later (13).

Peterson *et al.* (14) have reported the toxic effects of dehydroheliotridine (an optical isomer





of the stomach developed in rats injected with dehydroretronecine. Such ulcers invariably developed in the junctional zone. Hematoxylin and eosin; × 15. FIG. 5. Near complete depletion of the erythroid and myeloid elements of the bone marrow occurs within 72 hr in rats given dehydroretronecine. Hematoxylin and eosin stain; × 15. FIG. 6. This is an example of lymphocytic depletion in the spleen of a dehydroretronecine-treated rat within 48 hr. Hematoxylin and eosin stain; × 15.

of dehydroretronecine) in tissues. Many of the changes that were reported to occur in dehydroretronecine-injected animals are also evidenced in animals exposed to dehydroheliotridine. Perhaps the major difference in the toxic effects of these two pyrrole metabolites is apparent in the stomach where severe acute inflammation and gastric ulcerations were noted in the dehydroretronecine-treated animals, whereas only transient mucosal alterations in the stomach were recorded in animals that were given dehydroheliotridine.

The means by which dehydroretronecine interacts with the rapidly dividing cells to produce the inhibition of mitosis on one hand and a stimulation of neoplastic changes in tissues of the cervical area on the other hand remains to be established. The possibility exists that the tissue changes which occur in dehydroretronecine intoxication are a result of its interaction with cellular macromolecules. Culvenor *et al.* (15) and White and Mattocks (16) have shown that the pyrrolizidine alkaloids and their metabolic pyrroles are alkylating agents which are capable of producing an alteration in cellular nucleic acids. It is likely that dehydroretronecine acts in a similar manner.

Summary. When dehydroretronecine, a metabolite of the pyrrolizidine alkaloid monocrotaline, is given subcutaneously to rats at doses ranging between 90 and 250 mg per kg body weight, a conspicuous inhibition of cell mitosis occurs in the gastrointestinal tract. Hemorrhage and ulceration of the gastric mucosa were common in the animals that died or were sacrificed within 72 hr after treatment. In addition, the animals experienced splenic and thymic hypoplasia due primarily to the rapid depletion of their lymphocytic components. The bone marrow of these animals was practically devoid of all precursor cells within 48 hr after the administration of dehydroretronecine. There was also an inhibition of cell division in the seminiferous tubules. However, within 7 days, the cells in the gastrointestinal mucosa, spleen, thymus, bone marrow, and testes showed a decided improvement. These data indicate that the effects of dehydroretronecine are primarily limited to its inhibiting effect on mitotic division in rapidly dividing cells. Other lesions such as hepatic necrosis, pulmonary hypertension, and cor pulmonale which are caused by the parent alkaloid monocrotaline were not reproduced by dehydroretronecine.

1. Harris, P. N., Anderson, R. C., and Chen, K. K., J. Pharmacol. Exp. Ther. **75,** 78 (1942).

2. Newberne, P. M., and Rogers, A. E., Plant and Food for Man **1,** 23 (1973).

3. Hill, K. R., Rhodes, K., Stafford, J. L., and Aub, R., Brit. Med. J. **1,** 117 (1953).

4. Bras, G., Jelliffe, D. B., and Stuart, K. L., Arch. Pathol. **57,** 285 (1954).

5. Allen, J. R., and Chesney, C. F., Exp. Mol. Pathol. **17,** 220 (1972).

6. Butler, W. H., Mattocks, A. R., and Barnes, J. M., J. Pathol. **100,** 169 (1970).

7. Hsu, I. C., Allen, J. R., and Chesney, C. F., Proc. Soc. Exp. Biol. Med. **144,** 834 (1973).

8. Culvenor, C. C. J., Edgar, J. A., Smith, L. W., and Tweeddale, H. J., Aust. J. Chem. **23,** 1853 (1970).

9. Hayashi, Y., and Lalich, J. J., Proc. Soc. Exp. Biol. Med. **124,** 392 (1967).

10. Allen, J. R., Carstens, L. A., Norback, D. H., and Loh, P. M., Cancer Res. **30,** 1857 (1970).

11. Chesney, C. F., Allen, J. R., and Hsu, I. C., Res. Commun. Chem. Pathol. Pharmacol. **5,** 859 (1973).

12. Hsu, I. C., Shumaker, R. C., and Allen, J. R., Chem. Biol. Interact. **8,** 163 (1974).

13. Allen, J. R., Carstens, L. A., and Hsu, I. C., Cancer Res. (submitted).

14. Peterson, J. E., Samual, A., and Jago, M. V., J. Pathol. **107,** 175 (1971).

15. Culvenor, C. C. J., Downing, D. T., Edgar, J. A., and Jago, M. V., Ann. N.Y. Acad. Sci. **163,** 837 (1969).

16. White, I. N. H., and Mattocks, A. R., Biochem. J. **128,** 219 (1972).

Received June 18, 1974. P.S.E.B.M., 1974, Vol. 147.