15548

Effects of 2,4 - Dichlorophenoxyacetic Acid on Experimental Animals.*

NANCY L. R. BUCHER. (Introduced by Joseph C. Aub.)

From the Medical Laboratories, Collis P. Huntington Memorial Hospital, Harvard University, at the Massachusetts General Hospital, Boston, Mass.

2,4-Dichlorophenoxyacetic acid (2,4-D) is of biological interest because of its remarkable effects on plants. Its action is selective, not only with regard to different species of plants, but also with regard to the type of response in different plants and plant parts. It is a powerful growth stimulator in minute concentrations; actively growing plants are especially sensitive. Larger amounts produce morphologic changes which may occur at some distance from the point of application, and after an appreciable latent period, death of the plant ensues.[1-5]

In view of these properties it seemed worthwhile to investigate the effects of 2,4-D upon animals, with respect to toxicity, pharmacologic activity, and influence on normal and neoplastic growth. Plant hormones have previously been shown to inhibit the growth of transplantable tumors if applied directly to the tumor tissue,[6,7] but treatment of tumor-bearing animals by injection at a site remote from the tumor has been unsuccessful[7] except in rare instances.[8]

The sodium salt of 2,4-D was used in 1% solution in physiological saline, adjusted to approximate neutrality. Toxicity studies were carried out on young strain A male mice. The drug was injected either subcutaneously, intraperitoneally or intravenously and was found to be effective in the same general dose range by any of these routes. The drug has previously been reported to be nontoxic to animals and humans when administered orally.[4,5] In the present experiments the approximate LD_{50}, as determined by subcutaneous administration, was 280 mg per kg.

The injection of from 150 to 200 mg per kg at one time results in a myotonia-like syndrome that lasts from 8 to 24 hours or more in different animal species. The same signs have been produced in mice, rats, rabbits and dogs. Within a half to three-quarters of an hour following administration of the drug, the animal has ceased most spontaneous activity, and sits very still. However, he remains awake and alert. When he is now induced to make a sudden movement, such as a quick start, or a righting motion, spasm supervenes; his limbs spread out, and he falls and lies helpless, vainly trying to regain his footing. Opisthotonus may appear momentarily. The hind limbs are usually more noticeably affected than the fore limbs. If incited to continue his attempts to move, he will gradually recover. Motion is slow and awkward at first, but with continued effort approaches normal speed, smoothness, and control. After this, if the animal is allowed to remain still for 5 or 10 minutes, the initial syndrome recurs. In addition to the phenomenon of alleviation by exercise and exacerbation by rest, some animals exhibit a local hard knotty muscle spasm in response to a sharp percussive blow. Both features are characteristic of clinical myotonia. The diagnosis of myotonia has finally been established by myographic and electromyographic studies, now being done in conjunction with Dr. George Acheson of the Department of Pharmacology of the Harvard Medical School.

When larger doses (250 to 350 mg per kg)

* This is publication No. 622 of the Cancer Commission of Harvard University.

This work was aided by a grant from the American Cancer Society, Massachusetts Division, Inc.

1 Zimmerman, P. W., and Hitchcock, A. E., *Contr. Boyce Thompson Inst.*, 1942, **12**, 321.

2 Hamner, C. L., and Tukey, H. B., *Bot. Gaz.*, 1944, **106**, 232.

3 Marth, P. C., and Mitchell, J. W., *Bot. Gaz.*, 1944, **106**, 224.

4 Hildebrand, E. M., *Science*, 1946, **103**, 465.

5 Mitchell, J. W., and Marth, P. C., *Bot. Gaz.*, 1944, **106**, 199.

6 Kline, B. E., Wasley, W. L., and Rusch, H. P., *Cancer Research*, 1942, **2**, 645.

7 Kline, B. E., and Rusch, H. P., *Cancer Research*, 1943, **3**, 702.

8 Tanaka, A., and Tuboi, S., *Gann*, 1940, **34**, 354.

are given to mice there is an initial period of myotonia as previously described, followed by rapidly increasing sluggishness and reluctance to move; tails are rigid; attempts at righting bring out a coarse clonic tremor. Inertia deepens into coma. The mice now lie on their backs with the 4 feet in the air. Their respirations are almost imperceptible. They are flaccid, and cold to touch. This state may terminate in death after several hours, or several days. In a few instances, after 8 to 12 hours, the mouse may go through the same stages in reverse order, finally recovering completely and without residua. Dosages of this magnitude have not been administered to other animals.

Other toxic effects of the drug which have been noted following parenteral administration are: (1) an irritant action on the eyes and nasal passages of dogs, resulting in sneezing spells, and violent rubbing of the eyes, which develops after a delay of 24 hours or more (2) gastro-intestinal disturbances in dogs manifested by vomiting and increasingly severe anorexia (3) the production of diarrhea in many, but not all mice.

Thus far no clear-cut chronic syndrome has been produced by continued administration of one or 2 daily injections to mice (1/3 to 1/5 of the LD_{50} per day for 3 weeks to 3 months).

Postmortem studies have not revealed any striking features peculiar to the drug. In acutely intoxicated mice the liver, kidneys and spleen grossly are dark and mottled. The upper part of the intestine is filled with a bile-colored liquid which is sometimes blood-stained. The bladder is full, and a mass of dry, hardened feces is caked about the anus. Microscopic examination shows wide dilatation of the blood vessels of lungs, liver and kidneys. In mice receiving prolonged treatment the only abnormalities noted have been attributable to infection. There was moderate atrophy of the liver in one chronically-treated dog, whose serum alkaline phosphatase rose from 5.0 to 39.4 Bodansky units in 3 weeks. This dog entirely refused food during the last week of life.

The peripheral blood of chronically-treated mice has not varied significantly from the controls with respect to hemoglobin, red cell, white cell and differential counts.

Small doses have not affected the growth rate of young mice. Larger amounts (1/4 of the LD_{50} daily) have retarded growth, probably through reduction of food intake. Mice who have survived this amount for 3 months are still alert, active and apparently normal in other respects. Repeated injections have not influenced the growth rates of 2 transplantable mouse sarcomas (Sarcoma 180 and Sarcoma 37) to any notable degree. Mitotic counts performed on these tumors at varying intervals after a single injection of 200 mg per kg did not differ significantly from the control values. Mice undergoing daily injections of 1/3 of the LD_{50} have become pregnant, and borne apparently normal litters at the end of a normal gestation period.

Summary. Parenterally-administered 2,4-D produces temporary myotonia in mice, rats, rabbits and dogs following a single injection. Repeated injections have failed to elicit either a characteristic chronic syndrome or a striking histologic picture; nor have they altered the process of reproduction and development in the dose ranges tested. The slight retardation of the growth rate of young mice on large daily doses is probably a manifestation of reduced food intake. Neoplastic growth has not been inhibited.

The author is grateful to Dr. Joseph C. Aub for the suggestion of this problem and for encouragement and advice. The technical help of Amory Glenn is also greatly appreciated.