Attempts at Induced Agranulocytosis in Rats Using Dipyrone

By N. M. FERGUSON and ARTHUR F. NOVAK*

Dipyrone administered orally to rats at the rate of 50 and 100 mg. per day over a period of 8 weeks did not produce any abnormalities in the blood picture. In addition no visible differences could be observed in the rats receiving the drug over those of the control group. No sensitivity to the drug was observed.

AGRANULOCYTOSIS is a disease characterized by a marked change in the blood picture, particularly that of a reduction in the polynuclear leukocyte count. A great many substances have been suspected of causing agranulocytosis (1). Included in this list are industrial solvents, *e.g.*, benzene, toluene, naphtha, paints and paint removers, and heavy metals such as gold and lead and their salts. By far the greater number, however, consist of drugs. This list includes practically all of the different classes of drugs such as anti-infectives, sedatives, tranquilizers, cardiotonics, and analgesics. Since some analgesic drugs are used in large quantities for fever as well as for pain, the total number of doses prescribed annually is very great. As a result of the widespread use of these drugs even a very low incidence of agranulocytosis appears to be alarming (1).

Attempts at controlling the distribution of these drugs have resulted in their being limited to prescription use only. Since most of them are very effective in relieving pain and some in reducing fever, they often constitute the only means of producing these desired therapeutic effects.

One of the most effective analgesic drugs which is equally effective in reducing fever is dipyrone, a derivative of antipyrine. This substance has been suspected of being a causative agent in agranulocytosis (1). This tabulation indicates that prior to June 1963, eight cases of agranulocytosis were reported to the panel and an additional nine cases were cited in the literature where dipyrone was the only drug given in the 6-month period prior to the onset of the disease. Six other cases, four from direct reports and two from the literature, occurred in which dipyrone was given in conjunction with other drugs which are believed not to be associated with agranulocytosis. During the same period other cases were brought to light where dipyrone was given along with additional medication which in turn is not known to be innocent or in many cases is actually known to precipitate agranulocytosis. From July 1963 to July 1964, only two cases are reported where the only drug given during the previous 6-month period prior to the onset of the disease was dipyrone, whereas, four cases have been recorded during the same period where other drugs not known to be innocent of this reaction and four cases where drugs known to be toxic were administered. During these same periods, however, many other drug substances have been reported as being responsible for or associated with agranulocytosis and many of these in much higher incidence than dipyrone (1).

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* Present address: Louisiana State University, Baton Rouge.

TABLE I.—EXPERIMENTAL CONDITIONS

Group No.	Drug Received	Daily Dose, ml.	Days
1	None	0	30
2	Dipyrone[a]	0.5 (50 mg. dipyrone)	30
3	Dipyrone	1.0 (100 mg. dipyrone)	30

[a] The authors thank Savage Laboratories, Inc., Houston, Tex., for supplying dipyrone as Pyralgin Liquid used in this study.

Many attempts have been made to learn the exact mechanism of drug induced agranulocytosis. Kracke (2) found that clinical agranulocytosis could be produced in rabbits by subcutaneous injections of benzene. Lu *et al.* (3) reported that there was no reduction of blood cell count and hypoplasia of bone marrow was not observed in rabbits receiving daily administration of aminopyrine for 4 to 6 weeks even in the presence of a sensitizing agent such as busulfan.[1]

In view of the fact that dipyrone is prescribed widely for the relief of fever and pain and has been associated with the reported incidence of agranulocytosis, it was felt that further studies should be conducted with this substance. The present study, the first in a series, concerns attempts to induce agranulocytosis in rats with dipyrone.

EXPERIMENTAL

A series of 120 white rats, approximately 6 weeks old, having an average weight of 250-Gm., and equally divided as to sex, were selected for these experiments. The experimental design was planned so that the results could be analyzed statistically if this was necessary to interpret the results.

All of the rats were maintained since birth, and during the course of the entire experiment on Purina rat chow, which is a highly standardized commercial feed, fulfilling all of the nutritional requirements of rats for normal growth. Feed and water were available to the animals continuously.

The experiments were performed in an air-conditioned laboratory maintained at 72–74° F. Feed was added as required by replacing the used feed containers with clean filled containers of feed. Clean water was replaced daily. Records of feed consumption and body weight were kept for each animal.

At the outset of this investigation, the rats were separated into three groups of 20 males and 20 females each. Table I lists the experimental conditions.

Group *1* served as the control, while groups *2*

[1] Marketed as Myleran by Burroughs Wellcome & Co., Scarsdale, N. Y.

TABLE II.—AVERAGE RED BLOOD CELL COUNTS × 10^6/mm.^{3} FOR RATS RECEIVING DAILY ORAL DOSES OF DIPYRONE FOR 8 WEEKS

		Initial	Weeks 2	4	6	8
Control	Male	7.92	8.35	8.14	9.27[a]	9.16[a]
	Female	7.67	7.94	7.79	8.78[a]	8.93[a]
Dipyrone daily, 50 mg.	Male	8.10	7.88	8.54	8.61[a]	7.91[a]
	Female	8.59	8.03	7.95	8.52[a]	8.47[a]
Dipyrone daily, 100 mg.	Male	7.83	6.98	7.51	7.90[a]	8.28[a]
	Female	8.45	8.40	8.52	8.93[a]	8.76[a]

[a] Statistically similar at the 1% significance level both within the groups and also when compared to the change from initial averages.

TABLE III.—AVERAGE WHITE CELL COUNTS/mm.^{3} OF BLOOD FOR RATS RECEIVING DAILY ORAL DOSES OF DIPYRONE FOR 8 WEEKS

		Initial	Weeks 2	4	6	8
Control	Male	11,700	10,800	11,200	12,500	11,650
	Female	12,150	11,900	12,150	11,800	11,700
Dipyrone daily, 50 mg.	Male	12,000	12,100	11,350	10,700	9,950[a]
	Female	11,850	12,000	10,900	10,600	10,200
Dipyrone daily, 100 mg.	Male	11,400	10,600	9,850	9,800	9,550[a]
	Female	11,250	10,800	10,050	9,750	9,400[a]

[a] Statistically different at the 1% significance level when compared to the change from the initial averages.

TABLE IV.—DIFFERENTIAL BLOOD COUNTS × 10^3/mm.^{3} RECEIVING ORAL DOSES OF DIPYRONE FOR 8 WEEKS

	Neutrophils	Lymphocytes	Eosinophils	Monocytes	Basophils	Reticulocytes
Normal range	1.0–4.9	6.0–17.0	0.0–0.7	0.0–0.65	0.0–0.2	0.6–4.9
Control						
Male	1.2–3.2	8.0–12.2	0.2–0.4	0.2–0.6	0.0–0.2	0.7–3.8
Female	1.5–3.1	7.4–12.5	0.2–0.5	0.2–0.4	0.0–0.2	0.9–4.5
Dipyrone daily, 50 mg.						
Male	1.3–2.7	7.6–13.1	0.1–0.4	0.2–0.6	0.0–0.1	0.9–4.6[a]
Female	1.2–3.0	8.2–14.8	0.2–0.3	0.2–0.6	0.1–0.2	0.9–3.9[a]
Dipyrone daily, 100 mg.						
Male	1.2–2.3	7.7–12.6	0.2–0.5	0.2–0.5	0.1–0.2	0.8–4.2[a]
Female	1.1–2.6	7.1–13.9	0.2–0.2	0.3–0.6	0.0–0.2	0.7–4.1[a]

[a] Statistically similar at the 1% significance level when compared to the change from initial averages.

and *3* were administered daily doses of dipyrone according to the quantities listed in the above table.

Blood counts were made initially, and after every 2 weeks during the course of the experiment. Body weights were recorded and visual observations were made on each rat. None of the rats died during the course of the experiment.

PROCEDURES

Administration of Dipyrone.—The drug was administered orally each morning by employing a 1-ml. graduated pipet, and allowing the animal to drink the fluid as it was released slowly from the tip. No problems were encountered with this technique.

Blood Counts.—Since the tail is free from local circulatory change, it was used as the site of puncture. The tail was cleaned with ethanol and dried, and the tip of the tail was cut off with sterile surgical scissors. After the first few drops of blood were wiped away, the subsequent drops were obtained for red cell count, white cell count, and a blood smear for a differential count. Standard methods were employed for the counts.

RESULTS

All of the results obtained are presented in Tables II–IV. The total number of red and white blood cells are the average values. The individual variations in the population within the groups were too insignificant to have any biological importance.

DISCUSSION

The statistical treatment of the data was by the method of analyses of variance by the *F* test method. The variance of the average values of rats with dipyrone from an initial period to a period of 8 weeks was compared to that of initial averages of controls for the same period. Table IV shows the range of results for 20 rats in each series. It is to be noted that the highest and lowest values are within the normal ranges for each type of blood cell counted.

The weights and feed intake were the same for the control group as well as for the groups receiving the drug. No visual differences could be observed in the rats receiving the drug over those in the control group. Furthermore, the rats showed no sensitivity to the drug.

Four rats out of each series (*i.e.*, two males and two females) were checked at the end of 8 weeks and no abnormalities were found with respect to blood glucose, cholesterol, phospholipids, and urea nitrogen suggesting that no chemical changes resulted from the use of the drug.

SUMMARY AND CONCLUSIONS

In carefully controlled studies carried out over a period of 8 weeks, involving 120 rats, no statistically significant changes occurred in the blood picture with respect to red cell, white cell, and differential counts when varying amounts of dipyrone were administered orally.

No visual differences could be observed in the rats receiving the drug over those in the control group.

No rats died as the result of taking the drug.

No abnormalities were found with respect to blood glucose, cholesterol, phospholipids, and urea nitrogen on examining animals from each group suggesting that no chemical changes resulted from the use of the drug.

In this study using massive doses of dipyrone no indications of agranulocytosis were produced.

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Phytochemical Investigation of *Abies concolor*

By AYHAN ULUBELEN*, MARY E. CALDWELL, and JACK R. COLE

Extracts of the bark of *Abies concolor* have shown antitumor activity against the adenocarcinoma of the duodenum test system of the Cancer Chemotherapy National Service Center. One of the active materials appears to be a complex tannin. The isolation of the active fractions has been reported utilizing solvent extraction, column, paper, and thin-layer chromatography.

In a routine screen of Arizona and Mexico plants for antitumor activity, it was found that extracts of the bark of *Abies concolor* (Gordon and Glendinning) Hoopes were shown to have activity against the adenocarcinoma of the duodenum (7D1) test system of the Cancer Chemotherapy National Service Center, Bethesda, Md. The plant, also known as white fir, is a soft-wooded, resinous evergreen monoccious tree, 100–200 ft. tall, 3–6 ft. in trunk diameter. It is distributed from Wyoming west to Oregon and south into Baja California, Arizona, New Mexico, and Sonora, at elevations of 3000–10,000 ft.

The collection used in this study was obtained from upper Sabino Creek, 8000 ft. elevation, Santa-Catalina Mountains, Pima County, Ariz.[1]

EXPERIMENTAL

Preliminary Extraction.—The bark (3.5 Kg.) of *A. concolor* was extracted with chloroform–ethanol (1:1) in a Lloyd extractor for 2 days. After evaporation, this crude extract was submitted to the adenocarcinoma of the duodenum test system (7D1). The crude material showed a decrease in tumor size of approximately 86 and 84% at a dose of 400 and 200 mg./Kg., respectively (Table I).

Table I.—*In Vivo* Tumor Inhibition

	Dose, mg./Kg.	% T/C[a]
n-Hexane extract	175	32
	200	32
Brown powder	100	26
	200	11

[a] The criteria for activity is defined as being a % T/C (test/control) value of less than 42 in a satisfactory dose response test (1).

One kilogram of the crude material was extracted with 2000 ml. of *n*-hexane in a Soxhlet for 72 hr. The green *n*-hexane extract upon evaporation yielded 200 Gm. of gummy material. A brown powder was obtained in the Soxhlet. Both of the materials were active against the test system.

n-Hexane Extraction

Twenty-five grams of the green material obtained from the *n*-hexane extraction was extracted with 10 × 100 ml. of petroleum ether (36–60°). The petroleum ether was evaporated to dryness in a Rinco evaporator under reduced pressure. A light green residue (10 Gm.) was obtained.

Part A.—Five grams of this residue was chromatographed on a neutral alumina column (30 × 3 cm.) (Fisher certified reagent catalog No. A-950 Brockman, activity I, 80–200 mesh). The column

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* Present address: College of Pharmacy, University of Istanbul, Istanbul, Turkey.
[1] Identification confirmed by Robert Barr, Research Associate, College of Pharmacy, and Dr. Charles Mason, Curator of the Herbarium, Botany Department, University of Arizona, Tucson. A reference specimen was also deposited.