Leukopenia-Induction Capacity of 6-MP Palladium Complex in the Chick* (34074)

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(Introduced by H. O. Kunkel)

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Numerous investigations have been conducted on the immunosuppressive activity of 6-mercaptopurine (1–6). In addition, the following studies have been reported on the immunosuppressant properties of 6-MP derivatives: 2-amino-6-mercapto-8-phenylpurine (8-PTG), 6-mercapto-8-phenylpurine (8-PMP), (7–8); 8-aza-9-cyclopentylpurines (9); S-glycosides of 6-mercaptopurine (10); 6-hydroxyl-amino-9-β-D-ribofuranosylpurine (11–15); 2,6-dihydroxylaminopurine and its ribosyl derivative (16). Although many studies have been reported with respect to 6-MP and its many derivatives, there appears to be a dearth of information in the literature on the immunosuppressive activity of the metallic complexes of this compound. Previous reports from this laboratory were concerned with the effect of the platinum complex on skin homograft survival and the comparative leukopenia-induction capacity of this complex in young chicks (17, 18). The present investigation was designed to study the leukopenia induction effect of the palladium complex of 6-MP, originally synthesized and described in a paper devoted to the potential antiviral activity of a series of similar metallic complexes of 6-MP (19).

Experimental Procedure. Experimental animals. The White Leghorn cockerels (Hy-Line) were obtained from a commercial hatchery at 1 day of age. The experimental chicks were housed in brooders in a room maintained at a constant temperature of 72°F. Feed and water were provided *ad libitum.*

Treatment. The chicks were divided into control and treated groups of 25 each. The compounds were administered orally by capsule each day and dosage levels of 6-MP and the palladium complex were adjusted according to the weight gain or loss of each chick on an individual weight basis. The treated chicks were administered the compounds at the following daily dosage levels: equal levels of 1800 mg/kg of body weight, equimolar levels of 1800 (6-MP) versus 3168 mg/kg of body weight for the palladium complex. The drug weight dosage at the equimolar level was based on calculations which showed that the palladium complex contained approximately 50% of 6-mercaptopurine on a molar basis. Treatment was initiated at 5 days of age and continued through Day 10 of treatment. A total leukocyte count, after the method of Natt and Herrick (20), was taken daily at the same time each morning for each chick.

The procedure for preparation of the palladium complex was that of Kirschner (19). These workers proposed the empirical formula $Na_2[Pd(MP)_2Cl_2]$. (II) for the complexed compound. The analysis of variance and Duncan's multiple range tests (21) were used to evaluate the significance of difference in the leukocyte counts.

Results. The effect of 6-MP and its palladium complex on the leukocyte count at equal (1800 mg/kg body weight/day) and equimolar (1800 vs. 3168 mg/kg body

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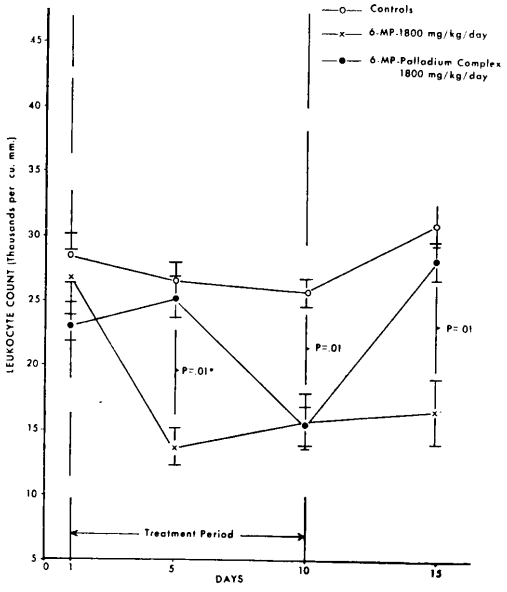

FIG. 1. Comparative effect of 6-MP and 6-MP-palladium complex (1800 mg/kg/day) on the average leukocyte count of 5-day-old chicks. Summary of three experiments; 75 birds each; *Highly significant.(* = Highly significant.)

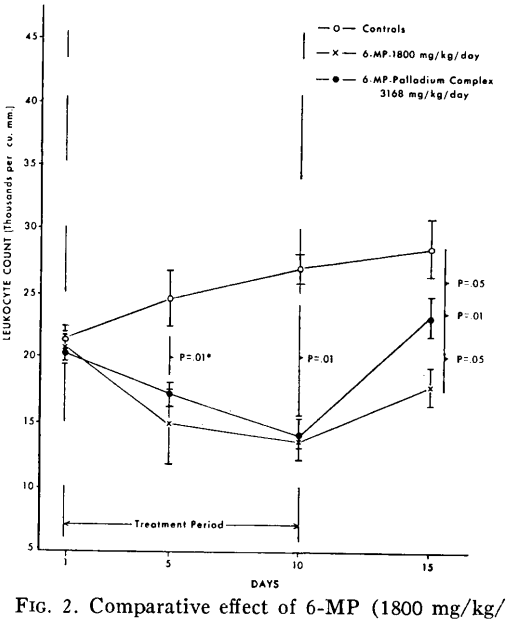

FIG. 2. Comparative effect of 6-MP (1800 mg/kg/day) and 6-MP-palladium complex (3168 mg/kg/day) on the average leukocyte count of 5-day-old chicks. Summary of four experiments; 75 birds each. *Highly significant.

weight/day) levels resulting from a series of experiments is shown in Figs. 1 and 2, respectively. Figure 1 shows that treatment of 5-day-old chicks with pure 6-MP caused a marked decrease in the average leukocyte count from 27,000 to 12,000, in contrast to an average of 25,000 observed after 5 days of drug administration for the chicks treated with the palladium complex. Significant reductions in the leukocyte counts of chicks in groups treated with 6-MP were observed at the end of the 5-day and 10-day treatment period (Figs. 1 and 2), $P = .01$. The leukocyte-suppressive effect of 6-MP was significantly greater than that of its palladium counterpart at the end of the 5-day treatment period (Figs. 1 and 2). After 5 days of treatment with the palladium complex, no significant change occurred in the leukocyte count. However, after 10 days of treatment a significant decrease was observed.

The leukocyte counts of chicks treated with equimolar dosage levels of 6-MP and the palladium complex (Fig. 2) were similar on Day 10 (the last day). However, the counts of both treated groups were significantly lower ($P = .01$) than those of the control groups. Leukocyte values after cessation of treatment (5 days post-treatment on Day 15) were significantly lower than that of the controls ($P = .05$) for both treated groups (Fig. 2). The relative capacity of both compounds with respect to leukocyte suppression was almost identical ($P = .01$). The rapid increase in leukocyte recovery during the 5-day post-treatment period for chicks treated with the palladium complex was again evident in this series of experiments. It should be noted that in the first series of experiments involving equal dosage levels the leukocyte suppressive activity of the palladium complex was not significantly manifested at Day 5 of treatment. However, administration of 3168 mg/kg/day of the palladium complex on an equimolar basis in the second series of experiments caused a significant decrease in the leukocyte count at the end of 5 days.

Figure 3 illustrates the comparative effect of 6-MP and the palladium complex on the growth rate of chicks to which the com-

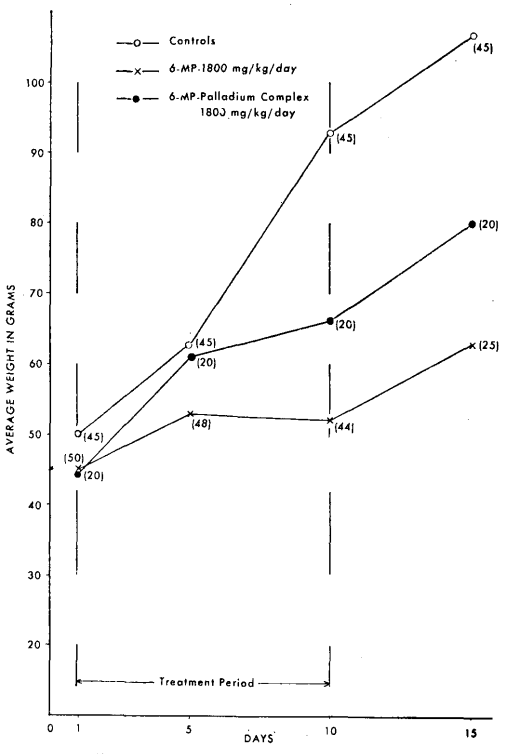

FIG. 3. Comparative effect of 6-MP and 6-MP-palladium complex (1800 mg/kg/day) on the average weight gain of 5-day-old White Leghorn cockerels. Summary of three experiments; 75 birds each. Surviving number indicated in parentheses.

pounds were administered for 10 days at equal (1800 mg/kg body weight/day) dosage levels. The chicks treated with 6-MP did not gain weight comparable to that exhibited by the chicks treated with the palladium complex. Mortality in chicks treated with 6-MP was excessive. No mortality was recorded for the groups to which the palladium complex had been administered. Weight gain and mortality of chicks subjected to equimolar dosage levels (1800 mg/kg/day of 6-MP vs. 3168 mg/kg/day of the complex) followed the pattern shown in Fig. 4 as was exemplified in previous experiments. It is of importance to note that the mortality of chicks treated with the complex was essentially the same as that occurring in the controls. Consequently, the desirable effect of leukocyte suppression with little or no effect on mortality was achieved by treatment with the palladium complex.

Discussion. The results of this investigation have confirmed the work reported in previous studies (17, 18) which demonstrated that 6-MP and its platinum complex were effective leukopenia-induction agents in the chick. According to the results, it appears that the palladium complex was as effective in its ability to induce leukopenia as both 6-MP and the platinum complex. The reduced toxicity of the palladium complex was apparent as was demonstrated by the comparative weight gain and absence of mortality exhibited by the chicks treated with the complex.

It has been shown that modification of the mercapto group of 6-MP to produce such derivatives as 6-alkyl-, 6-aryl-, 6-purinyl, and 6-imidazolyl mercaptopurines caused retention of the immunosuppressive activity, and in some cases, effected an enhancement of this immunosuppressant property (4–6, 22). The activity of the S-substituted 6-MP has

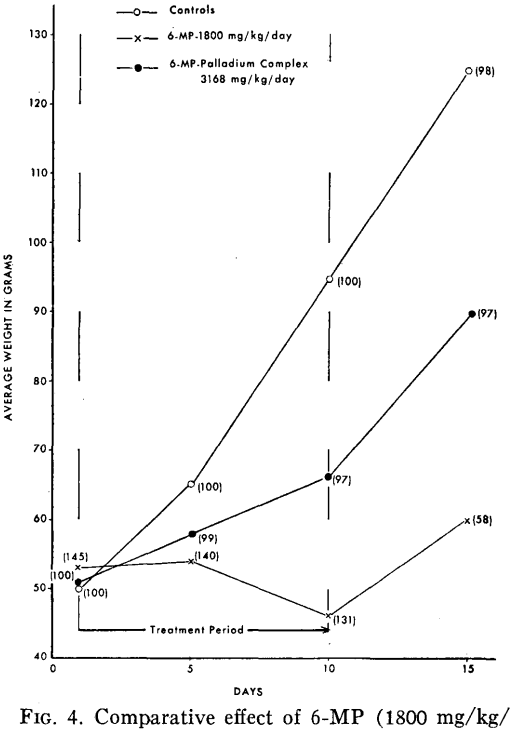

FIG. 4. Comparative effect of 6-MP (1800 mg/kg/day) and 6-MP-palladium complex (3168 mg/kg/day) on the average weight gain of 5-day-old White Leghorn cockerels. Summary of four experiments; 75 birds each. Surviving number indicated in parentheses.

been shown to be manifested only after *in vivo* hydrolysis or reduction with liberation of the free mercapto group (23). Consequently, the metallic-ligand bonding (platinum and palladium) at the sixth position of 6-MP appear to have caused a reduction in the toxicity of 6-MP without loss of immunosuppressive activity. Furthermore, the palladium complex appears to possess the desirable property of leukocyte suppression without causing the excessive mortality usually associated with 6-MP treatment and other immunosuppressive agents. The work reported herein justifies the continued study of these complexes and emphasizes the need for further studies on the pharmacodynamics of the immunosuppressive process. The specific role of the metallic substitution in the reduced toxicity of these compounds should also be investigated.

Summary. A series of experiments was conducted with 5-day-old White Leghorn (Hy-Line) cockerels. Six-mercaptopurine and a palladium complex of this compound were administered orally to 5-day-old chicks at equal dosage levels on a milligram basis for 10 days. At equal dosage levels, the leukopenia induction capacity of the two compounds was essentially the same at the end of the 10-day treatment period. The palladium complex was less toxic, as demonstrated by greater weight gain and lower mortality. At equimolar dosage levels, results were similar to those observed when evaluation of equal dosage levels was conducted.

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