Effect of 6-MP and 6-MP–Palladium Complex on Serum Uric Acid Levels in the Chick[1] (37052)

R. W. LEWIS,[2] M. J. DENNIS, AND A. T. BURRS
(Introduced by H. O. Kunkel)
Texas A&M University - Prairie View Experiment Station, Prairie View, Texas 77445

Uric acid levels in the blood and urine are frequently elevated in leukemia. During the treatment of this condition with X-rays, steroids or purine antimetabolites, hyperuricemia is accentuated and increased quantities of uric acid are excreted in the urine (1–5). That secondary gout is a potential occurrence in any acquired hyperuricemic state has also been well documented (6). Several investigators have reported on studies designed to counteract this undesirable side effect. The hypoxanthine isomer, 4-hydroxypyrazolo(3,4-*d*)pyrimidine (allopurinol) was found to decrease urinary uric acid excretion in mice and man (7–11). As a result of previous studies (12, 13) designed to determine the leukopenia-induction capacity of platinum and palladium complexes of 6-mercaptopurine (6-MP), it seemed feasible to ascertain the effect of the palladium complex on serum uric acid levels in young chicks.

Experimental Procedure. Experimental animals. Day-old white leghorn cockerel (Hy-Line) chicks, obtained commercially, were housed in brooders in a room maintained at a constant temperature of 25°. Feed and water were provided *ad libitum*.

Treatment. The chicks were divided into control and treated groups of 25 each. In the first series of experiments, the chicks treated with 6-MP received an average dosage of 1800 mg/kg of body weight/day. The chicks treated with the palladium complex were administered an average dosage of 3168 mg/kg/day. Treatment was initiated at 5 days of age and continued until Day 10. Details of the treatment schedule were previously published (13).

In the second series of experiments, the chicks treated with 6-MP received an average dosage of 900 mg/kg of body weight/day. The palladium complex was administered to a group of chicks at an average dosage of 3168 mg/kg of body weight/day. A group of chicks initially treated with 900 mg/kg/day of 6-MP for 5 days were subsequently treated with 3168 mg/kg/day of the palladium complex for the remaining 5 days of the 10-day period. All chicks were given the compounds orally in No. 4 gelatin capsules.

Uric acid determinations of serum aliquots pooled from the blood of five chicks in each group killed by decapitation were made according to the method of Bittner (14) at the following intervals: (a) 5 days after treatment; (b) at the end of the 10-day treatment period; (c) 5 days posttreatment. The procedure for the preparation of the palladium complex was that of Kirschner *et al.* (15). The analysis of variance and Duncan's multiple range test (16) were used to evaluate the significance of difference in serum values.

Results. Figure 1 depicts the average serum uric acid concentrations of the various experimental groups analyzed as a result of four experiments involving 75 chicks each. After 5 days of treatment with pure 6-MP, a significant elevation in the serum uric acid level (9.88 ± 0.12 mg/100 ml) was observed when compared with the uric acid level of the controls (6.13 ± 0.25 mg/100 ml) and that of chicks treated with the palladium complex (5.04 ± 0.07 mg/100 ml). Uric acid values

[1] Supported in part by the Texas Agricultural Experiment Station, Texas A&M University, College Station, TX and the Robert A. Welch Foundation.

[2] Present address: Department of Biochemistry & Biophysics, Texas A&M University, College Station, TX 77843.

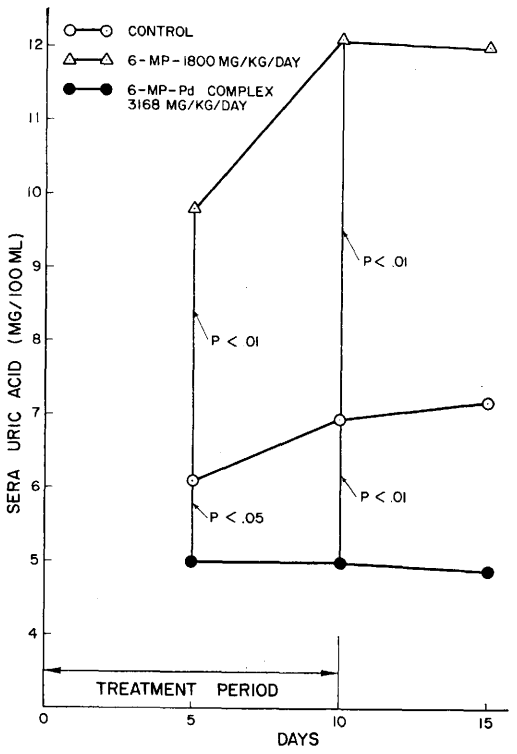

FIG. 1. Effect of 6-MP (1800 mg/kg/day) and 6-MP–palladium complex (3168 mg/kg/day) on average serum uric acid concentration (mg/100 ml of blood) following 5- and 10-day treatment periods and fifth day posttreatment. Summary of four experiments: 75 birds each.

recorded at the end of the 10-day treatment period indicated that the palladium complex evoked a significant suppression of uric acid concentration (5.00 ± 0.54 mg/100 ml) as opposed to a value of 12.14 ± 0.17 mg/100 ml for chicks which received pure 6-MP. The comparable value for the controls was 6.95 ± 0.63 mg/100 ml. Cessation of treatment for a period of 5 days evidenced no apparent effect on the uric acid levels of either of the treated groups. There was a nonsignificant elevation of the uric acid concentration for the controls over the same 5-day posttreatment period.

A second series of four experiments involving 100 chicks each was carried out to determine if the high level of uric acid concentration caused by treatment with 6-MP could be counteracted by administration of the palladium complex for a 5-day treatment period. Figure 2 illustrates the effect of 900 mg/kg/day of pure 6-MP administered to chicks for 5 days and the subsequent effect of 5-day treatment of the same chicks with 3168 mg/kg/day of the palladium complex. There were no significant differences between the values for any of the treated chicks at Day 5. However, the values for all treated groups were significantly higher than that of the controls. According to the data recorded for Day 10, administration of 3168 mg/kg/day of the palladium complex for 5 days to chicks previously treated with pure 6-MP caused a significant depression in serum uric acid concentration to a value of 8.2 ± 0.17 mg/100 ml which was not significantly different than that of either the controls (8.4 ± 0.36 mg/100 ml) or those chicks which received the palladium complex for the entire 10-day period. It should be noted that treatment of chicks with 900 mg/kg/day of pure 6-MP for a continuous period of 10 days resulted in a significant elevation in the uric acid level for this second series of experiments in a manner comparable to that ef-

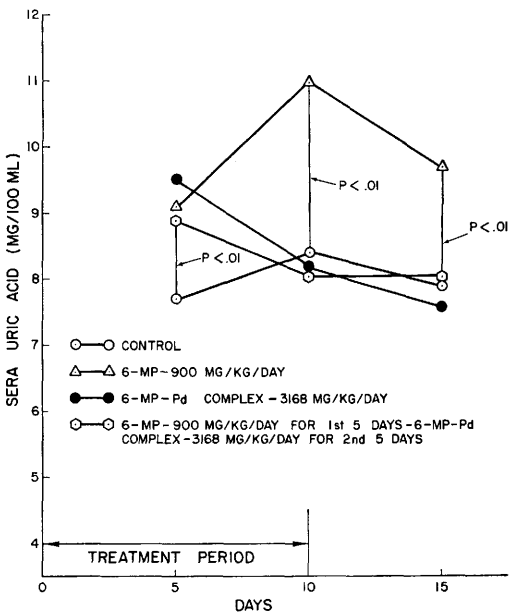

FIG. 2. Effect of 6-MP (900 mg/kg/day) on serum uric acid concentration (mg/100 ml of blood) following 5 days of treatment, subsequent treatment with 6-MP–palladium complex (3168 mg/kg/day) for 5 days and fifth day posttreatment. Summary of four experiments: 100 birds each.

fect observed in the first series of experiments.

Following the 5-day posttreatment period, serum uric acid concentrations were decreased in all groups of treated chicks. The level of serum uric acid (9.7 ± 0.48 mg/100 ml) in chicks treated with pure 6-MP remained significantly higher than those of the controls (7.9 ± 0.21 mg/100 ml) and chicks treated with the palladium complex for the entire 10-day treatment period (7.6 ± 0.17 mg/100 ml). It appeared that subsequent palladium complex treatment of chicks previously treated with pure 6-MP achieved a reduction of uric acid synthesis which continued to be manifested at the end of the 5-day posttreatment period.

Macroscopic examination of the internal organs of 6-MP-treated chicks revealed hepatic necrosis, massive liver discoloration and enlargement of the gallbladder. Chicks treated with the palladium complex alone revealed none of the previously described conditions. Chicks first treated with 6-MP and subsequently treated with the palladium complex evidenced only a slight discoloration of the liver in a minority of all chicks examined. These observations were made with the controls being utilized as standards.

Discussion. The results of this study indicating that the antimetabolite, 6-MP, causes an increase in serum uric acid concentration of chicks substantiate the findings of other investigators. According to Gerbrandy, Hellendoom and Lockerbol (1), Sandberg, Cartwright and Wintrobe (2), Krakoff (3), Watkin and Silver (4), Philips *et al.* (5), very large quantities of uric acid are synthesized and excreted during treatment of leukemia with antimetabolites. The studies reported herein showed that chicks treated with the palladium complex had lower serum uric acid levels than those given pure 6-MP. Such data suggests a decreased rate of uric acid synthesis and/or an increased rate of excretion. Krakoff (3) stated that not all antimetabolites caused a rise in uric acid production. It was postulated that some antimetabolites may also block purine synthesis at a stage prior to that of uric acid formation and their administration is followed by a fall rather than a rise in uric acid production. Since the serum uric acid values observed as a result of this investigation were less in palladium complex-treated chicks, it is highly possible that the complex elicited a suppression of uric acid synthesis by the mechanism proposed by Krakoff (3). Since xanthine oxidase (avian xanthine dehydrogenase) catalyzes the conversion of hypoxanthine and xanthine to uric acid, a more specific possibility is the inhibition of the enzyme by the palladium complex of 6-MP. Stetten and Fox (17) have shown that small concentrations of copper inactivates this enzyme. Recently published reports (18, 19) have indicated that uric acid synthesis in the chick can be blocked by reduction of xanthine dehydrogenase activity with 4-hydroxypyrazolo(3,4-*d*)pyrimidine (allopurinol) and *o*-diazeacetyl-L-serine (azaserine). These studies confirmed results obtained in an earlier investigation (20) in which Krakoff and Meyer (10) were able to reduce the amount of uric acid excreted by chicks fed allopurinol. However, these workers could not account for this decrease with an increase in xanthine and hypoxanthine and thus postulated the presence of an unidentified end product.

These results should be confirmed bv further studies with other species in which leukemia has been experimentally induced. The possible value of the complexes in the treatment of hyperuricemia in gout should also be investigated.

Summary. A series of experiments was conducted with 5-day-old white leghorn (Hy-Line) cockerels. 6-Mercaptopurine and a palladium complex of this compound were administered orally to 5-day-old chicks at 1800 and 3168 mg/kg/day, respectively, for 10 days. At the end of the treatment period, the serum uric acid level of chicks treated with pure 6-MP was significantly elevated. In contrast, treatment with the palladium complex caused a significant decline in serum uric acid which was less than that evidenced by the controls. In a second series of experiments, it was demonstrated that elevation of uric acid concentration caused by treatment with 900 mg/kg/day of pure 6-MP for 5 days could be significantly depressed by subsequent treatment with 3168 mg/kg/day of the palladium complex.

1. Gerbrandy, J. H., Hellendoom, B., and Lockerbol, H., Brit. J. Cancer **12**, 275 (1959).

2. Sandberg, A. A., Cartwright, G. E., and Wintrobe, M. M., Blood **11**, 154 (1956).

3. Krakoff, I. H., Med. Clin. N. Amer. **45**, 521 (1961).

4. Watkin, D. W., and Silver, R. T., Amer. J. Med. **24**, 638 (1958).

5. Philips, E. S., Sternberg, S. S., Hamilton, L., and Clarke, D. A., Ann. N. Y. Acad. Sci. **60**, 283 (1954).

6. Wyngaarden, J. B., *in* "The Metabolic Basis of Inherited Disease" (J. B. Stanbury, J. B. Wyngaarden and D. S. Fredickson, eds.), 2nd ed., p. 705. McGraw-Hill, New York (1966).

7. Hitchings, G. H., Ann. Rheum. Dis. **25**, 601 (1966).

8. Elion, G. B., Ann. Rheum. Dis. **25**, 608 (1966).

9. Rundles, R. W., Wyngaarden, J. B., Hitchings, G. H., Elion, G. B., and Silberman, H. R., Trans. Ass. Amer. Phys. **76**, 126 (1963).

10. Krakoff, I. H., and Meyer, R. L., J. Amer. Med. Ass. **193**, 1 (1965).

11. Balis, M. E., Krakoff, I. H., Berman, P. H., and Dancis, J., Science **156**, 1122 (1967).

12. Lewis, R. W., Rubin, B. A., Davis, J. E., and Woods, J. J., Proc. Soc. Exp. Biol. Med. **128**, 857 (1968).

13. Lewis, R. W., Mack, R. W., Dennis, M. J., and Woods, J. J., Proc. Soc. Exp. Biol. Med. **131**, 1219 (1969).

14. Bittner, D., Halls, S., and McCleary, M., Amer. J. Clin. Pathol. **40**, 423 (1963).

15. Kirschner, S., Wei, Y. K., Francis, D., and Bergman, J. G., J. Med. Chem. **9**, 369 (1966).

16. Duncan, D. B., Biometrics **11**, 1 (1955).

17. Stetten, M. R., and Fox, C. L., J. Biol. Chem. **161**, 333 (1945).

18. Weir, E., and Fisher, J. R., Biochim. Biophys. Acta **222**, 556 (1970).

19. Horn, G. W., and Featherston, W. R., Poultry Sci. **51**, 882 (1972).

20. Krakoff, I. H., and Meyer, R. L., J. Pharmacol. Exp. Ther. **149**, 417 (1965).

Received Sept. 8, 1972. P.S.E.B.M., 1973, Vol. 142.