The Effect of a Folic Acid Antagonist, A-Methopterin, on the Level of the Circulating Eosinophils in Humans

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WHILE THE MECHANISM of action of the folic acid antagonists is not yet clearly understood, there is evidence of a relationship between these drugs and the adrenal cortex. Higgins observed hyperplasia of the adrenal glands with atrophy of the spleen and thymus in rats on autopsy, following the administration of A-Methopterin. Higgins and Woods reported that removal of the rats' adrenal glands, which had enlarged with the administration of a folic acid antagonist, decreased the changes these drugs usually produced in the spleen, thymus, peripheral blood and bone marrow of normal rats. Decreased values of the urine corticosteroids in humans with acute leukemia, treated with Aminopterin, were observed by Hanlon and associates and Schoenbach and co-workers. Gubner et al. have reported recently that, with the administration of Aminopterin, there occurred remission of symptoms and signs of articular and periarticular inflammatory reactions in rheumatoid arthritis and psoriasis. These above reports indicate the possibility that the folic acid antagonists may act via the adrenal gland. Gubner and co-workers, however, feel that the beneficial effect of Aminopterin in rheumatoid arthritis and psoriasis is not mediated by stimulation of the adrenal cortex, but probably by inhibition of connective tissue reactivity with suppression of inflammatory and proliferative tissue response.

The reports of Hills, Thorn and Roche and their associates have established clearly the intimate relationship between the level of the circulating eosinophils and the activity of the adrenal cortex. A fall in the blood eosinophil level, similar to that produced with the adrenocorticotropic hormone (ACTH), was seen in the "alarm reaction" in animals following stress. This change in both cases was due apparently to a stimulation of the adrenal cortex. It is the 11–17 oxysteroid hormones of the adrenal cortex which produce, by some unknown mechanism, the fall in the level of the circulating blood eosinophils.

Before the direct relationship between the 11–17 oxysteroid hormones of the adrenal and the eosinophil level had been described, decreases in the level of the eosinophils had been reported in a variety of acute clinical conditions such as infections, starvation, injections of foreign protein, surgery, etc. Observations on the eosinophil count in man by Fisher et al. showed a significant fluctuation or diurnal variation in the eosinophil counts of fasting and non-fasting individuals.
The purpose of this study was to investigate further the possible relationship between the folic acid antagonists and the adrenal gland. Since a direct relationship between the adrenals and the blood eosinophil level has been suggested, the eosinophil level was chosen to investigate the effect of a folic acid antagonist.

**Material**

Twenty-six persons were selected for study in this investigation. Of this group, 10 were used as untreated controls and 16 were subjected to medication with A-Methopterin (4 amino-N'-methylpteroylglutamic acid),* one of the folic acid antagonists. Four females and six males, all hospital personnel, ranging in age from 20 to 45 years, were chosen for the control group. These persons were in apparent good health, with no history of allergy or chronic disorders. During the period of this study, these subjects remained healthy, took no medication and continued routine activities. The 16 persons to whom A-Methopterin was administered were hospital patients who volunteered for this study. There were 6 women and 10 men in this group, ranging in age from 29 to 66 years. Some of these patients were chronically ill and others were on the orthopedic wards. During the study these patients remained afebrile and without evidence of acute infection or illness.

**Procedure**

Blood eosinophil counts and total white blood cell counts were done daily or every second day on both the control group and the medication group during the period of this study. The eosinophil counts, performed according to the method of Randolph,17,18 were taken at approximately the same time each day. Counts were done immediately upon withdrawal of the blood specimen. Several control counts were obtained prior to drug administration in the drug group, and then daily, or every second day, during A-Methopterin administration. The drug was given in daily oral doses of from 20 to 35 mg. until the appearance of signs of toxicity. The period of drug administration varied from six to fifty-six days.

The signs of toxicity which were looked for were ulcerations of the buccal mucosa, fall in the total white blood cell count, nausea, vomiting or diarrhea. One terminal case of carcinoma of the breast, with metastases to the lungs and bones, developed ulcerations of the buccal mucosa, a low white blood cell count, vomiting and diarrhea, while receiving A-Methopterin. In this case the drug was discontinued and the citrovorum factor (Leucovorin) was administered. None of the other 15 patients became seriously or uncomfortably toxic.

**Results**

Figure 1 shows the curves of the daily eosinophil counts on the normal controls. There was moderate irregular fluctuation from day to day and marked variation occurred in one instance. The maximum fluctuation was 33 per cent.

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* The A-Methopterin and Leucovorin used in this investigation were furnished through the courtesy of the Lederle Laboratories Division of the American Cyanamid Company, Pearl River, N. Y.
with the exception of the one control. In this individual the counts varied as much as 60 per cent, the cause of which was not determined.

Figures 2 and 3 show the curves of the daily eosinophil counts on the patients to whom A-Methopterin was administered. There was a varying period of moderate daily fluctuation, followed by a sharp downward trend. The marked fall in the eosinophil counts in this group of patients occurred either a few days before or at the time of toxicity from the drug. In this series of cases, 12 (Cases
1 to 12, fig. 2) showed sharp reductions in their eosinophil counts following the administration of A-Methopterin. Four cases (Cases 13 to 16, fig. 3) showed no reductions in their counts following A-Methopterin administration. In 10 of the 12 cases (Cases 1 to 10, fig. 2), reductions in the eosinophil counts of over 50 per cent were observed. In 2 cases (Cases 11 and 12), at the time of toxicity, moderate decreases of 37.1 per cent and 40.9 per cent respectively were noted. Six patients (Cases 1, 2, 3, 4, 8 and 11) showed a fall in the eosinophil count before clinical evidence of toxicity. Two patients (Cases 5 and 10) showed neither clinical nor laboratory evidence of A-Methopterin toxicity, although decreases of 79 per cent and 52.3 per cent in the eosinophil counts occurred. These latter patients were both discharged from the hospital before toxicity occurred and failed to return daily for the completion of the study.

Four patients (Cases 13, 14, 15 and 16, fig. 3), to whom A-Methopterin was administered, showed no definite pattern in their eosinophil curves. Case 13 was a patient who entered the hospital in delirium tremens with multiple simple fractures. The eosinophil count in this patient increased progressively, then decreased sharply to the control level at the time toxic manifestations were observed. Cases 14, 15 and 16 were patients who complained of slight nausea and malaise following varying periods of time on A-Methopterin and refused to continue medication. Whether these were definite manifestations of toxicity is questionable.

With the variation in the eosinophil counts, there was no corresponding variation in the daily total white blood cell counts.

**DISCUSSION**

Twelve of 16 cases to whom A-Methopterin was administered showed definite and progressive reductions in their daily eosinophil counts, which did not return
to the base line at the time of or prior to A-Methopterin toxicity, as compared with the group of 10 normal controls who were untreated and showed only moderate daily fluctuation in their eosinophil curves. Three of the 16 patients in whom no decrease in the eosinophil counts was recorded, exhibited only questionable evidences of A-Methopterin toxicity. As 75 per cent of the patients who received the drug showed a definite reduction in the level of their eosinophils, this study would suggest that A-Methopterin in some way stimulates the adrenal cortex or some other mechanism to cause a decrease in the blood eosinophil level. The variations in the response obtained may be related to the activity of the adrenal cortex of the individual patients or to some other unknown factor.

During this study it was observed that younger patients in good condition tolerated larger doses of A-Methopterin for longer periods than did the older, debilitated patients.

SUMMARY AND CONCLUSIONS

1. Daily eosinophil counts were obtained on a group of 10 normal individuals. This control group showed a moderate daily fluctuation in the count, with only 1 exception.

2. Daily eosinophil counts were obtained on 16 chronically ill or convalescent patients to whom A-Methopterin was administered. The eosinophil curves of 10 of these patients showed a fall of over 50 per cent at or prior to A-Methopterin toxicity. In 1 case, the count increased then decreased at toxicity to the initial level. In 3 cases definite evidence of toxicity was not demonstrable and the eosinophil level did not decrease.

3. Six patients showed a fall in their eosinophil counts before clinical evidence of A-Methopterin toxicity. Three patients showed a fall in their eosinophil counts of 75 per cent at the time of A-Methopterin toxicity.

4. Twelve of 16 cases (75 per cent), to whom A-Methopterin was administered showed definite falls in the curves of daily eosinophil counts. That cortisone and the adrenocorticotropic hormone do likewise suggests, but is not evidence, that the mechanisms are related.

REFERENCES

7 HILLS, A. G., FORSHAM, P. H. AND FINCH, C. A.: Changes in circulating leukocytes in-
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