Effect of Methyltestosterone on the Nucleic Acid Metabolism of Rat Bone Marrow Cells[1] (35914)

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There is considerable information about the hormonal control of erythropoiesis mediated by erythropoietin and sexual steroids (1–3).

The action of erythropoietin at the bone marrow level has been established with some degree of confidence; however, the action of steroids on the same tissue is poorly understood. With regard to erythropoietin, numerous reports have shown that the hormone acts on bone marrow cells probably by stimulating erythroid precursors to proliferate and stem cells to differentiate (4). Since a reasonable correlation can be established between action of erythropoietin and changes in nucleic acid metabolism, the progress of the erythropoietic process can be followed by an analysis of the nucleic acid metabolism of bone marrow (1, 5).

Some authors have postulated that the erythropoietic effect of certain androgens like testosterone is an indirect one, due presumably to increased production of endogenous erythropoietin by the kidney (3, 6, 7). Other authors have proposed that testosterone increases the amount of a factor, which after interaction with some specific plasma proteins generates erythropoietin (8). Naets and Wittek (2) and Reisner (9) have provided evidence indicating that the erythropoietic effect of testosterone may be exerted, not only through the production of erythropoietin, but also directly on some specific target cells of the bone marrow.

This communication describes the effect of β-methyltestosterone on nucleic acid metabolism of rat bone marrow, following the experimental approach proposed by Perretta and Tirapagui (1) and Perretta (5). It is shown below that short pulses of methyltestosterone stimulate RNA and DNA metabolism of bone marrow tissue as measured by the incorporation of ^{14}C-formate into RNA and DNA bases.

Materials and Methods. Experiments were performed using normal and polycythemic male rats of the strain A × C (wt, 150–170g), that were injected intravenously with 5 μCi of ^{14}C sodium formate (sp act 10 mCi/mmole).

In this study polycythemic rats have been used since they provide a rather clean system for detecting the erythropoietic effect of different hormones (7, 10). Polycythemic rats, prepared by the method of transfusion, show a marked suppression of erythropoiesis, due to a low rate of differentiation of stem cells into erythroblasts (11), and have a slow RNA metabolism (1). Polycythemia was produced in rats by injecting intraperitoneally one dose of 7 ml of a suspension of isologous red blood cells in saline. Hemoglobin was measured (12), as an index of the level of polycythemia, 2 days after transfusion by using the blood obtained from the tail vessels. Hemoglobin was 14–16 g/100 ml in normal rats and 19–21 in polycythemic animals.

Methyl-testosterone (Abbot, 5 mg/100 g of body wt) suspended in 0.9% saline was injected intraperitoneally 2 hr before the rats were killed. When erythropoietin was assayed, each rat received an intravenous injection of 6 units of erythropoietin extract (14 units/mg) 2 hr before death. This extract was prepared according to the method of

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Lowry and Borsook (13) and assayed by the method used by Rudolph and Perretta (14).

Two hours after tracer injection rats were killed by bleeding under ether anesthesia; and bone marrow was removed from the femur and tibia using 0.9% NaCl. The separation of nucleic acid bases was performed according to the technique described by Smellie *et al.* (15), which was modified by the use of high voltage electrophoresis instead of paper chromatography, in order to gain in time and resolution of base separation. The electrophoresis was performed at 3,000 V and 140 mA, using 0.02 *M* phosphate buffer, pH 8.0. Samples were run in Whatman 3 MM paper for 3 hr and spots were localized by using an ultraviolet lamp (252 m/μ) and then compared with standards. For the determination of specific activity, spots from the strip were cut and eluted with 0.1 *N* HCl. The ^{14}C bases were counted at infinite thinness in a gas flow counter. The optical density was measured at the proper wavelength for each base in a Perkin-Elmer spectrophotometer. Specific activity was expressed (cpm/μmole of base).

Results. The results of a series of *in vivo* experiments, on the effect of methyltestosterone on the incorporation of ^{14}C-formate into the bases of nucleic acid are shown in Table I. Notice that compared to control rats, animals that have been subjected to a 2 hr pulse of methyltestosterone show a considerable increase in the specific activities of both RNA-adenine and DNA-thymine. A 2 hr pulse was used since previous *in vivo* experiments had shown that a linear relationship exists between time of exposure to methyltestosterone and its effects on nucleic acid metabolism of the rat bone marrow.

TABLE I. *In Vivo* Effect of Methyltestosterone on the Incorporation of ^{14}C-Formate into RNA-adenine and DNA-thymine of Normal Rat Bone Marrow After 2 hr.

	Sp act (cpm/μmole of base)	
	RNA-adenine	DNA-thymine
Control (6)[a]	480 ± 120[b]	105 ± 10
Methyltestosterone	1078 ± 230	195 ± 8

[a] Parentheses indicate the number of animals used in the experiment.

[b] Standard error.

TABLE II. *In Vivo* Effect of Methyltestosterone and/or Erythropoietin on the Incorporation of ^{14}C-Formate into RNA-adenine and DNA-thymine of Bone Marrow from Polycythemic Rat after 2 hr.

	Sp act (cpm/μmole of base)	
	RNA-adenine	DNA-thymine
Control[a] (4)[b]	125 ± 8[c]	102 ± 7
Methyltestosterone (5)	921 ± 50	206 ± 17
Erythropoietin (4)	257 ± 17	105 ± 6
Methyltestosterone + erythropoietin (5)	1249 ± 63	222 ± 20

[a] Polycythemic animals were used as controls.

[b] Parentheses indicate the number of animals used in the experiments.

[c] Standard error.

Table II shows the effect of methyltestosterone and/or erythropoietin on the uptake of ^{14}C-formate by the nucleic acid bases of bone marrow cells from polycythemic rats. It appears clear that methyltestosterone induces a marked increase in the specific activity of RNA-adenine, which is seven times greater than that displayed by the polycythemic control. Erythropoietin produces a two- to threefold increase of the specific activity of RNA-adenine. When both hormones are assayed simultaneously, an additive effect is observed on RNA-adenine specific activity. Table II also shows the effect of methyltestosterone and/or erythropoietin on the DNA-thymine metabolism. There is a marked difference between the effect produced by the hormones on the DNA-thymine metabolism compared with that produced on RNA-adenine metabolism. Thus, methyltestosterone produces a 100% increase in DNA-thymine specific activity while erythropoietin does not have any appreciable effect. When both hormones are assayed simultaneously, the effect is similar to that produced by methyltestosterone alone.

Discussion. Many of the studies dealing with the erythropoietic effect of androgens,

specially those related with testosterone, have been performed by using long pulses of the hormone and by giving successive injections and thus maintaining a high blood level of the hormone. This experimental model has indicated that the erythropoietic effect of androgens is mediated by endogenous erythropoietin (2, 8).

However, there is a scarcity of data relating to a possible direct action of androgens on bone marrow not mediated by erythropoietin. We have studied the effect of testosterone on bone marrow through an analysis of the incorporation of a labeled precursor into the nucleic acid, searching for an early effect of the hormone. *In vivo* experiments reported in this paper have demonstrated that a short pulse of methyltestosterone increases in the incorporation of ^{14}C-formate into both of the nucleic acids. In contrast, erythropoietin appears to influence RNA metabolism in the first 2 hr.

The possibility that testosterone acts directly on the bone marrow, through a mechanism which does not involve an increase in the production of endogenous erythropoietin, can be inferred from our results. If the action of testosterone on bone marrow cells were related to a change in the rate of production of erythropoietin, one would expect to find no effect of the hormone on the DNA-thymine (1), but our results show that methyltestosterone produces a change in DNA-thymine specific activity. The work of Fried and Gurney (7), who have demonstrated that normal rats injected with testosterone do not exhibit any detectable increase of plasma erythropoietin during the first 24 hr, gives additional support to the idea of an erythropoietin-independent effect of methyltestosterone on bone marrow cells.

Since the present study was done using whole marrow, it is not known whether the observed effect of androgen is related to erythropoiesis and which cells are responding to the hormone. (Erythropoietin sensitive cells? granulocyte precursors? etc.)

Summary. The effect of methyltestosterone on the nucleic acid metabolism of rat bone marrow cells was studied 2 hr after injection of the hormone. Methyltestosterone increased the uptake of ^{14}C-formate into both RNA and DNA bases in both normal and transfusion-induced polycythemic rats. When the hormone was assayed in polycythemic rats a sevenfold increase in the specific activity of RNA-adenine was observed. The specific activity of DNA-thymine was two times greater than that of polycythemic controls. Under the same conditions erythropoietin produced a change only in the specific activity of RNA-adenine. The simultaneous injection of both hormones produced an effect on RNA-adenine that was a summation of the effect of each of the hormones separately. However, under the same conditions, the effect of both hormones on the DNA-thymine was that produced by testosterone alone. These findings suggest that the effect of testosterone on the nucleic acid metabolism of rat bone marrow is not mediated by erythropoietin.

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