

be separated from the crude extract by short-path vacuum distillation at 50°C and 0.1 mm for 4 h. Thin-layer chromatography indicated the presence of at least 3 major components in the distillate (Table III). The component present in largest quantity (fraction I) has been identified as lactic acid which is a known major component of sweat and has been shown to be repellent to mosquitoes when tested on the skin<sup>4</sup>. This component was isolated by preparative thin-layer chromatography and identified by comparison of its thin layer and vapor phase chromatographic behavior and IR-spectral characteristics with those of an authentic aqueous solution of lactic acid subjected to similar evaporative drying in vacuo. Such samples exhibit 2 major peaks on vapor phase chromatography – presumably due to lactic acid and lactic anhydride. The other 2 major repellent components of sweat (II and III) have not yet been identified but appear to be less polar hydroxy carbonyl compounds from their IR-absorption spectra. Vapor phase chromatography indicates that these fractions are each composed of a major (216°/12 min and 155°/6.5 min) and 2 minor components.

Addition of water to the ether-extracted lactic acid (fraction I) or to authentic acid reduced the repellency in the olfactometer to marginal levels. Therefore, the relative significance of lactic acid as a nullifying factor in the attractiveness of bulk sweat samples is uncertain. However, the presence of varying amounts of lactic acid on the skin surface may affect the degree of attractiveness of individuals – particularly in combination with other factors such as the amount of moisture on the skin. For instance, anhydrotic humans have greatly decreased attraction to *Aedes* mosquitoes. On addition of water to their skin, normal levels of attractancy return<sup>5</sup>. It is becoming increasingly apparent that the attractiveness of humans to mosquitoes is governed by a complex interaction of numerous endogenous factors including heat,

moisture, carbon dioxide, lipids, acids and possibly unknown substances in trace amounts<sup>2,6,7</sup>.

**Zusammenfassung.** Es wird gezeigt, dass menschliche Schweißabsonderungen verschiedener Versuchspersonen auf weibliche Moskitos (*Aedes aegypti*) verschieden attraktiv sind. Mit Diethyläther (pH 1) lassen sich im Schweiß sowohl «anlockende» wie auch «abschreckende» Bestandteile extrahieren. Für den «repellent effect» sind teilweise gleiche Mischungen von Milchsäure und Milchsäureanhydrid verantwortlich. Zwei weitere, noch nicht identifizierte Substanzen zeigten im Zwei-Kammer-Olfaktometer identische «repellent» Wirkungen.

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## Effect of Erythropoietin on Nucleic Acid Metabolism from Polycythemic Rat Bone Marrow

Many facts appear to be in favour of the control activity of the erythropoietin on erythropoiesis by means of the regulation of the metabolism of RNA<sup>1-4</sup>. However, the lack of uniformity in the erythropoietic tissues, which consists of cells with markedly different functional properties, makes the problem very difficult to investigate at the molecular level. The difficulties of interpreting the experimental data prompted us to search for conditions more suitable for understanding the biochemical mechanisms involved in the erythropoietic process. One of these experimental conditions is the establishment of metabolic patterns of the nucleic acids from hematopoietic tissues under the effect of erythropoietin. In this way, it is possible to obtain valuable biochemical information in order to know more about the mode of action of the hormone. The metabolic patterns are determined by measuring nucleic acid metabolism with the criterion of the incorporation of some labelled precursors into the macromolecules. Formate is a suitable precursor for the biosynthesis of nucleic acid because it can be incorporated to a good extent by RNA and DNA<sup>5</sup>.

This communication describes experiments in which different metabolic patterns of bone marrow from normal, polycythemic (hypertransfusion) and polycythemic rats treated with erythropoietin were studied.

The materials and methods were as previously described<sup>1</sup>. Other conditions are given in the Table.

The (<sup>14</sup>C)formate incorporation patterns are shown in the Table. It is clear that different patterns are obtained under the 3 experimental conditions. The metabolism of nucleic acid from normal marrow follows a pattern already demonstrated<sup>1</sup>. In polycythemic rats the nucleic acid metabolism decreased 2- to 4-fold, especially RNA adenine, while the specific activity of thymine was not changed to such an extent. The injection of erythropoietin produced an increase of 2- to 3-fold in acid-soluble adenine and RNA, whereas no observable effects are produced in DNA. These observations corroborate our previous findings in spleen and bone marrow from polycythemic mice

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Incorporation in vivo of ( $^{14}$ C)formate into acid-soluble fraction (ASF), RNA and DNA bases of the bone marrow of normal, polycythemic (hypertransfusion) and polycythemic rats treated with erythropoietin

Animal condition	ASF	Whole RNA		DNA		
	Adenine	Adenine	Guanine	Thymine	Adenine	Guanine
Normal	2839 $\pm$ 232 (7)	1266 $\pm$ 203 (9)	759 $\pm$ 106 (7)	298 $\pm$ 39 (7)	1022 $\pm$ 286 (8)	769 $\pm$ 116 (6)
Polycythemic	947 $\pm$ 165 (8)	284 $\pm$ 94 (8)	379 $\pm$ 90 (6)	126 $\pm$ 22 (5)	247 $\pm$ 68 (8)	289 $\pm$ 83 (6)
Polycythemic + erythropoietin	2725 $\pm$ 325 (7)	638 $\pm$ 116 (9)	999 $\pm$ 101 (7)	133 $\pm$ 33 (5)	312 $\pm$ 105 (7)	440 $\pm$ 66 (6)

Figures give specific activity (means  $\pm$  S.E. of the mean) in cpm/ $\mu$ mole of bases obtained from the sources indicated. Figures in parentheses give the No. of animals. Male rats of the strain A  $\times$  C weighing 140–160 g were used for the studies. Polycythemia was produced by injecting i.p. 1 dose of 7 ml suspension of isologous red blood cells. Hemoglobin was determined as an index of the level of polycythemia<sup>11</sup> 2 days after the transfusion. Hemoglobin % in g was 14–16 (normal) and 22–26 (transfused). 10  $\mu$ C of ( $^{14}$ C)formate were injected i.v. 2 h before sacrifice. The animals treated with erythropoietin were injected i.v. with 10 U of erythropoietin contained in plasma obtained from anaemic rats for 3 h. Nucleic acids were isolated and analysed according to a method already described<sup>1</sup>. ( $^{14}$ C)formate specific activity: 18 mC/mg (New England Corp.).

in which the hormone only affects the activity of RNA during the first 10 h of action<sup>3</sup>.

Under polycythemic conditions in which the erythropoietic process is almost suppressed<sup>6,7</sup> a single injection of erythropoietin induces a differentiation wave that proceeds from the stem cells to the reticulocytes. In this synchronous line of differentiated erythroid cells, it is possible to evaluate the metabolism of nucleic acid of these cells.

From our results an important conclusion can be drawn; that it is the tremendous nucleic acid activity of the erythropoietic cells which are few in number in comparison with the others. When red serie cells disappear the metabolism of the nucleic acid of the whole tissue drops very sharply, while it starts to increase when the cells appear under the action of the erythropoietin. In terms of nucleic acid metabolism, we have suggested<sup>8</sup> that the early activity provoked by erythropoietin could reflect the molecular action of the differentiation process, while the later increase of DNA can be interpreted as an indirect consequence of the action of erythropoietin.

From the operational point of view, our results indicate that bone marrow from polycythemic rats behaves in a similar fashion as normal bone marrow<sup>1</sup>, differing with the behaviour of spleen from polycythemic mice in which the metabolism of spleen nucleic acid is markedly depressed (10- to 20-fold) and increased after the action of the erythropoietin (3- to 4-fold)<sup>5</sup>. Spleens of transfused mice seem more appropriated for these studies than does rat bone marrow line in the same conditions.

The first molecular action of the erythropoietin known today is the early stimulation of the metabolic activity of RNA. It has been demonstrated at the morphological level that erythropoietin induces the differentiation of the stem cells to proerythroblasts, a mechanism that is inhibited by actinomycin D<sup>8</sup>. At this early stage, it is

natural to expect that a DNA-dependent RNA synthesis occurs. LINGREL<sup>9</sup> has reported an interesting finding in connection with this. He showed the presence of a high molecular weight RNA, labelled at early times in bone marrow from anaemic rabbits. Fractions of this RNA have the characteristics of messenger RNA. TORELLI et al.<sup>10</sup> suggest that the information for protein synthesis must be prepared in earlier stages of the erythropoietic process, this idea being consistent with the finding of an early synthesis of unstable RNA in normal human erythroblasts.

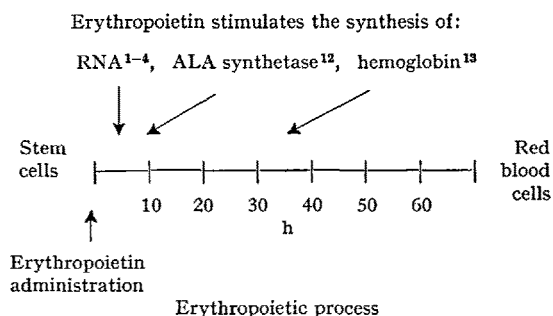
If we correlate the biological trilogy, DNA  $\rightarrow$  RNA's  $\rightarrow$  protein (enzymes) with the molecular effects induced by erythropoietin (see Scheme) in the erythropoietic process reported by several authors<sup>1-4,12,13</sup> we could suggest that the possible molecular action of the hormone might be at some level in the transcription stage.

Direct proof of this idea will be reported later on<sup>14</sup>.

**Resumen.** El efecto de la eritropoyetina sobre el metabolismo de los ácidos nucleicos de la médula ósea de rata policitémica por transfusión se caracteriza porque solamente estimula el metabolismo del ácido ribonucleico a tiempos cortos de acción (4 h). Se compara el efecto que produce la hormona sobre los ácidos nucleicos de médula ósea de rata policitémica y bazo de ratón policitémico. Se discute el posible rol bioquímico de la hormona.

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