It is also more potent and more selective than haloperidol, but shorter acting.

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## Effect of Erythropoietine on Incorporation of Formate labelled with Carbon-14 into the Nucleic Acids of Normal Rabbit Tissues *in vitro*

It has previously been shown that the blood and urine of rabbits suffering from hæmolytic anæmia contain a mucoprotein humoral factor, hæmopoietine or erythropoietine, which on injection into normal animals stimulates erythropoiesis[1]. The present experiments were undertaken to find out what effect this factor might have on nucleic acid biosynthesis in normal rabbit tissues *in vitro*. A crude preparation of erythropoietine was obtained from the urine of animals made anæmic with phenylhydrazine. The urine was brought to *p*H 4·5 with hydrochloric acid, and 4 volumes of ethanol were added. After standing overnight at 4° C. the precipitate was centrifuged down, washed with ethanol and ether, and dried. The dry powder was extracted with a small volume of Krebs–Ringer bicarbonate buffer. The protein concentration of this 'anæmic urine extract' was determined by the biuret method. A 'normal urine extract' was similarly prepared from the urine of normal rabbits. The effects of both extracts on the incorporation of formate labelled with carbon-14 into the nucleic acids of bone marrow, spleen and liver *in vitro* were determined by the methods of Smellie *et al.*[2].

Table 1. Incorporation of $^{14}$C-formate* into Nucleic Acid Bases of Normal Rabbit tissues *in vitro*

| Additions | Product analysed | Specific activity (counts/min./μmole) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Bone marrow | | Spleen | | Liver | |
| | | RNA | DNA | RNA | DNA | RNA | DNA |
| Nil | Adenine | 2,495 | 1,183 | 426 | 188 | 128 | 406 |
| | Guanine | 539 | 631 | 167 | 113 | 100 | 102 |
| | Thymine | | 24,500 | | 317 | | 1,026 |
| 'Normal urine extract'† | Adenine | 980 | 536 | 489 | 159 | 240 | 486 |
| | Guanine | 335 | 395 | 202 | 108 | 167 | — |
| | Thymine | | 16,200 | | 438 | | 675 |
| 'Anæmic urine extract'† | Adenine | 3,080 | 963 | 3,750 | 662 | 3,055 | 734 |
| | Guanine | — | 374 | 1,205 | 372 | 1,220 | 425 |
| | Thymine | | 28,400 | | 730 | | 671 |

Incubation time, 4 hr. * Isotope concentration, 5 μc./ml. † Concentration of extracts, 2·3 mgm. protein/ml. RNA, ribonucleic acid; DNA, deoxyribonucleic acid.

The results obtained are shown in Table 1. In agreement with results previously reported[2], the pattern of incorporation in normal bone marrow is characterized by the very high activity of the deoxyribonucleic acid thymine relative to the purines of both ribonucleic acid and deoxyribonucleic acid. In contrast, in spleen and liver deoxyribonucleic acid the activity of the thymine is generally only slightly greater than that of the purines. In previous papers[2,3] it has been argued that these observations may indicate that bone marrow, unlike the other tissues, may be incapable of synthesizing purine nucleotides fast enough to meet its own requirements. Bone marrow also differs from spleen and liver in its response to the urine extracts. The 'anæmic extract' produces virtually no effect on it, while the 'normal extract' tends to depress the activity of the nucleic acid purines and to a lesser extent, of the deoxyribonucleic acid thymine. In liver and spleen, on the other hand, the 'normal extract' has little or no effect, while the 'anæmic extract' causes a dramatic increase in the activity of the purines in both nucleic acids. In spleen, though not in liver, the anæmic extract also increases the activity of the deoxyribonucleic acid thymine.

These observations suggest that *in vivo* erythropoietine may stimulate *de novo* nucleic acid synthesis in liver and spleen, but not in bone marrow. However, Smellie *et al.*[4] have found that, in the rabbit, hæmolytic anæmia results in increased incorporation of $^{32}PO_4$ into the nucleic acids, not only of spleen, but also of bone marrow. An explanation of this latter observation may perhaps be found in the relationship between liver and bone marrow. Lajtha and Vane[5] have shown that *in vivo* hepatectomy greatly diminishes incorporation of $^{14}$C-formate into the nucleic acid purines of rabbit bone marrow. Clearly, therefore, the liver must play some part in the synthesis of the purines which the bone marrow requires (for example, for nucleic acid synthesis), but which it cannot synthesize for itself. It is therefore quite possible that erythropoietine might stimulate erythropoiesis in the bone marrow indirectly by acting on the liver. The increased incorporation of $^{14}$C-formate into liver nucleic acid purines produced by erythropoietine in the present experiments might be a reflexion of such action.

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