archaeological and paleontological work. Detailed reports on all aspects of the Museum's Medicine Creek field project are in preparation.


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Manuscript received October 11, 1951.


## Antagonistic Action of Uracil, Thiouracil, and Thiourea on Reticulocyte Ripening


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Granulocytopenia and agranulocytosis are well-known complications of the treatment of thyrotoxicosis with the drugs of the thiourea group. A few cases of anemia have been observed (*1–3*). A slight drop in erythrocyte count has been found by Goldsmith, Gordon, Finkelstein, and Charipper (*4*) in rats poisoned by thiourea. According to Weinglass, Williams, Bissel, and Peters (*5*), a decrease of hemoglobin level is evidenced in rats receiving thiouracil. We have previously shown that thiourea, thiouracil, and methylthiouracil inhibit reticulocyte ripening *in vitro* (*6*). Besides, an antagonistic action of thiouracil and uracil on bacterial growth has been observed by Strandskov and Wyss (*7*) and Wolff and Karlin (*8*). On the basis of these observations, it appears quite possible that the inhibiting activity of thiouracil and its derivatives on blood cell ripening and regeneration could be due to an interference with the normal metabolism of uracil. This hypothesis seems to be confirmed, in the particular case of red blood cells, by the experiments described in this paper. The experimental results show a competitive antagonism of uracil, thiourea, and thiouracil on the ripening of young erythrocytes (reticulocytes) *in vitro*.

The observation of reticulocyte ripening *in vitro* has already made possible an experimental investigation of various toxic and metabolic factors concerned with erythropoiesis (*6, 9, 10*). Our technique for the study of reticulocyte ripening has been fully described elsewhere (*9, 11*); it will be summarized here.

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Blood samples containing 10% heparin-glucose solution (heparin 200 mg %, glucose 1% in NaCl 0.9%) are drawn from adult dogs made anemic by repeated blood withdrawals, the hemoglobin level being maintained around 6–8 g %. The dogs are kept in good health and isolated; worms and other parasites are eliminated. Standard diet includes bread, 250 g; potatoes, 250 g; meat, 150 g; yeast extract, 2 g; and powdered iron, 40 mg daily. The animals submitted to chronic hemorrhage anemia show a high reticulocyte level in the peripheral blood (50–200/1000 RBC) and a normal reticulocyte ripening.

Basal solutions used are prepared as follows:

| | | |
|---|---|---|
| 1) | Thiouracil | 200 mg |
| | NaOH 1/10 *N* | 17 ml |
| | Buffer at pH 7.34 (Na2HPO4 1/5 *M*-citric acid 1/10 *M*) | 3 " |
| 2) | Thiouracil | 100 mg |
| | NaOH 1/10 *N* | 7.5 ml |
| | Buffer at pH 7.34 (Na2HPO4 1/5 *M*-citric acid 1/10 *M*) | 12.5 " |
| 3) | Thiourea, 0.5% and 1% in NaCl 0.9% | |
| 4) | Uracil, 5000, 500, 50, 25, 12.5, 5, 2.5 μg/ml NaCl 0.9% | |

The above solutions are added to aliquot portions of the blood samples, in a proportion of 10–20%, in order to obtain concentrations of thiouracil or thiourea of 350–2000 μg/ml blood. The concentrations of uracil in the blood are between 500 and 0.25 μg/ml. Control experiments are performed without thiourea, thiouracil, or uracil supplementation. The blood samples are incubated at 37° C for 6–8 hr. Reticulocyte counts are made at the beginning and at the end of the incubation period, according to our usual technique (*12*) (observation on dark field of *dried* smears after post-vital staining with brilliant cresyl blue), in various parts of each smear, and on a minimum basis of 5000 RBC. Reticulocyte ripening is evidenced by the decrease in reticulocyte number during incubation of the blood. The higher the decrease, the faster the ripening of young erythrocytes. Experimental results are depicted in Tables 1 and 2.

The experiments demonstrate that reticulocyte ripening is slowed or blocked by either thiourea or thiouracil at a range of concentrations between 350 and 2000 μg/ml blood. The block of ripening is evidenced by a reduced decrease of reticulocyte number during incubation of the blood.

The block of ripening is lifted by uracil at considerably lower concentrations (less than 5 μg/ml). After simultaneous supplementation with thiouracil and uracil, or thiourea and uracil, the reticulocyte ripening is normal and proceeds at the same rate as in control experiments. A competitive antagonistic action of thiourea, thiouracil, and uracil on red cell ripening is evidenced.

On the basis of the above-described results it appears very probable that the inhibition of erythropoiesis by thiourea and thiouracil is due to an interference of these drugs with normal uracil metabolism. It should be remembered that uracil is a constituent

TABLE 1

ANTAGONISTIC ACTION OF THIOURACIL AND URACIL ON RETICULOCYTE RIPENING

| Experiments | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dogs | | NLP4 | NLP4 | L10 | NLP4 | L10 | L10 | L10 | NLP4 | NLP4 | NLP4 |
| Incubation time (hr) | | 6 | 6 | 6½ | 6 | 6 | 7 | 6 | 7 | 8 | 7 |
| No. reticulocytes/1000 RBC before blood incubation | | 124 | 77 | 130 | 96 | 131 | 154 | 156 | 75 | 79 | 69 |
| After blood incubation | Conc μg/ml | | | | | | | | | | |
| | Thiouracil / Uracil | | | | | | | | | | |
| | 0 / 0 | 78 | 57 | 89 | 62 | 92 | 120 | 105 | 51 | 54 | 43 |
| | 350 / 0 | 104 | 71 | | | | | | | | |
| | 350 / 500 | 77 | 51 | | | | | | | | |
| | 1000 / 0 | | | 115 | 87 | 116 | 135 | 149 | 69 | 72 | |
| | 1000 / 500 | | | | 68 | | | | | | |
| | 1000 / 50 | | | 78 | 72 | | | | | | |
| | 1000 / 5 | | | 81 | 62 | 88 | 102 | 106 | | | |
| | 1000 / 2.5 | | | | | | 102 | 108 | 42 | 43 | |
| | 1000 / 1.25 | | | | | | 111 | 116 | | 41 | |
| | 1000 / 0.5 | | | | | 99 | 123 | 107 | | 49 | |
| | 1000 / 0.25 | | | | | | | 136 | 44 | | |
| | 2000 / 0 | | | | | | | | | | 69 |
| | 2000 / 5 | | | | | | | | | | 44 |
| | 2000 / 0.25 | | | | | | | | | | 58 |

TABLE 2

ANTAGONISTIC ACTION OF THIOUREA AND URACIL ON RETICULOCYTE RIPENING

| Experiments | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dogs | | NLP4 | L10 | L10 | L10 | B7 | NLP4 | L10 | L10 | L10 | BB3 |
| Incubation time (hr) | | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7½ | 7 |
| No. reticulocytes/1000 RBC before blood incubation | | 127 | 70 | 127 | 128 | 69 | 92 | 63 | 107 | 151 | 73 |
| After blood incubation | Conc μg/ml | | | | | | | | | | |
| | Thiourea / Uracil | | | | | | | | | | |
| | 0 / 0 | | 50 | 81 | 87 | 44 | 57 | 44 | 76 | 115 | 55 |
| | 500 / 0 | | | 119 | 111 | 60 | 77 | 56 | 96 | | |
| | 500 / 5 | | | 82 | 85 | 40 | 57 | 41 | 77 | | |
| | 500 / 2.5 | | | 83 | 88 | | | 37 | 83 | | |
| | 500 / 1.25 | | | 73 | | | | | 77 | | |
| | 500 / 0.5 | | | 111 | 106 | | | 40 | | | |
| | 500 / 0.25 | | | 125 | 104 | 43 | 54 | 51 | 81 | | |
| | 1000 / 0 | 112 | 64 | | | | | | | 147 | 72 |
| | 1000 / 5 | 79 | | | | | | | | 116 | |
| | 1000 / 2.5 | | 48 | | | | | | | | 56 |
| | 1000 / 1.25 | | 48 | | | | | | | 117 | |
| | 1000 / 0.5 | | 48 | | | | | | | 141 | |
| | 1000 / 0.25 | 67 | 60 | | | | | | | 137 | 60 |

of ribonucleic acids concerned with anabolic processes and present in the reticulocytes (*13–15*).

The inhibition of granulocyte regeneration may be caused by the same process. That antithyroid activity might also be the result of a disturbance of uracil metabolism, according to the hypothesis of Poupa (*16*) and Lauter and Stewart (*17*), is possible, but remains to be proved.

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Manuscript received September 17, 1951.