# Effect of Aminopterin on Radioactivity of Rat Liver- and Intestinal-Ribonucleic Acid after $C^{14}$ Formate Injection.* (21006)

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During the course of experiments on the metabolism of $C^{14}$ formate in rats the effect of aminopterin injection on the incorporation of the carbon of formate into ribonucleic acid purines was determined. The experiments were conducted somewhat differently from those of Goldthwait and Bendich(1) and an increase in the radioactivity of the liver ribonucleic acid purines was found instead of a decrease as reported by those authors. Skipper, Mitchell, and Bennett had previously made similar experiments on mice(2) and likewise had reported a reduction of formate incorporation following aminopterin injection.

*Methods.* For the present experiments 6 male Sprague-Dawley albino rats weighing 90-110 g each were employed. Two of these animals served as normal controls while two were injected with 100 $\mu$g of aminopterin daily for 4 days. The remaining two served as inanition controls and were pair-fed with the aminopterin treated animals. All animals received a purified diet similar to that used by Kelley *et al.*(3). *White blood cell* counts were made by standard procedures on each of the animals several times during the course of the experiment. The results of the counts are recorded in Table I.

Sixty-eight hours after the beginning of aminopterin treatment each animal received by intraperitoneal injection 10 $\mu$c (10 $\mu$ moles) of $C^{14}$ sodium formate. Nineteen hours later all animals were sacrificed and samples of liver and small intestines taken for analysis.

TABLE I. White Blood Cell Numbers of Controls and Aminopterin-Treated Albino Rats.

| Time after aminopterin treatment, hr | Controls: Food *(ad lib)* | Controls: Pair-fed | Aminopterin-treated |
|---|---|---|---|
| | Thousands/$\mu$l | | |
| -24 | 12.9 | 14.3 | 15.8 |
| | 15.6 | 16.3 | 19.8 |
| +37 | 12.5 | 23.4 | 10.5 |
| | 16.4 | 17.8 | 15.1 |
| 66 | 16.1 | 20.2 | 8.4 |
| | 19.6 | 16.5 | 9.6 |
| 87 | 21.3 | 17.8 | 5.9 |
| | 17.3 | 16.0 | 1.6 |

The tissues were homogenized in 5 volumes of water and 5 ml of the homogenate used for the analyses, which were carried out in duplicate. The fractionation procedure of Schneider(4) as modified by Ogur and Rosen (5) was employed. Following removal of the acid-soluble materials with trichloroacetic acid and the phospholipids with alcohol and alcohol-ether, the ribonucleic acid was extracted with cold 1 N $HClO_4$. Most of the $HClO_4$ was removed by precipitation with KOH and aliquots of the supernatant fluid evaporated to dryness on glass plates and counted with an end-window Geiger tube and scaler. Counts were corrected for background

TABLE II. Radioactivity of Intestinal and Liver Ribonucleic Acids (RNA) and Proteins 19 Hr after $C^{14}$ Formate Injection into Control and Aminopterin in Treated Albino Rats.

| Treatment | Specific activity: cts/min./$\mu$Mol RNA P*: Liver | cts/min./$\mu$Mol RNA P*: Intestine | cts/min./mg protein: Liver | cts/min./mg protein: Intestine |
|---|---|---|---|---|
| None, controls | 10.0 | 413 | 24.5 | 42.8 |
| | 3.5 | 294 | 14.5 | 20.2 |
| Pair-fed " | 15.0 | 325 | 24.0 | 24.0 |
| | 13.0 | 426 | 36.5 | 37.0 |
| Aminopterin inj. | 231 | 231 | 38. | 22.3 |
| | 205 | 233 | 32.6 | 21.7 |

* Values for nucleic acid phosphorus calculated using a molar absorption of 10000 at 260 m$\mu$.

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and, where necessary, for self absorption.

The desoxyribonucleic acid was extracted with 1.0 N $HClO_4$ at 70°. This extract, unlike the ribonucleic acid extract, was found to be grossly contaminated with radioactivity which could not be attributed to the purines or the thymine. The residual protein was washed with water, alcohol and ether and the dried powdered material counted on glass plates. Data on the protein radioactivity are included in Table II for comparison with the RNA counts.

As shown in Table II the aminopterin treatment appeared to reduce somewhat the formate incorporation into the intestinal ribonucleic acid. These results are in accord with those of Goldthwait and Bendich(2). On the other hand the liver RNA in the aminopterin-treated animals was 12-60 times more radioactive than that of the controls. Confirmation of this result was obtained in another experiment with 4 additional animals. Furthermore, the purines from the hydrolyzed RNA were isolated by precipitation as silver salts. The regenerated purines contained substantially all of the counts originally found in the RNA extracts. That the animals were affected by the aminopterin in the expected way is indicated by the lowered white blood cell counts shown in Table I.

Two possible explanations for the results on liver RNA suggest themselves. If aminopterin interferes with the formation of endogenous formate, the liver formate "pool" may be much reduced in size after a few days of treatment. Consequently, the dilution of the added radioactive formate would be much smaller in the treated animals and the specific activity of newly-formed purines correspondingly high. In addition to this effect it is possible that the reduction of active formate to methyl groups is much more sensitive in the rat to aminopterin inhibition than is purine formation. Such an effect might well divert active formate from methyl group or serine synthesis to purine synthesis. A result similar to this has been found in rabbit bone marrow *in vitro*(6).

It appears that whole animal experiments such as these will not be satisfactory until more complete knowledge of pool sizes of all intermediates, as well as net synthesis data are available.

*Summary.* Treatment of albino rats four times with 100 $\mu$g of aminopterin during a 68-hour period resulted in a severely lowered white blood cell count. The treated animals showed a reduced uptake of $C^{14}$ formate in intestinal ribonucleic acid while their liver ribonucleic acid showed a markedly increased specific activity as compared with controls.

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