CCLXXXI. THE ISOLATION OF METHYLMALONIC ACID FROM RAT URINE.

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IN isolating the metabolic products of anthracene from rat urine considerable difficulty was encountered in separating the glycuronic acid in a pure condition. Large volumes of acidified urine from rats fed as previously described [Boyland & Levi, 1935] were extracted with ether for 72 hours. The glycuronic acid was extracted by the ether but on account of its low solubility in ether crystallized out in the extract. The ether appeared to contain other acids but very little glycuronic acid. The ethereal extracts were concentrated to a semi-crystalline syrup which was drained on a porous plate. The major part of the solid was found to be crystallizable from hot toluene and proved to be methylmalonic acid. Between 200 and 500 mg. of the acid were obtained from each litre of urine. The acid crystallized in plates, m.p. 126–128°. It was soluble in water, alcohol and acetone, and the solutions were optically inactive. It did not react with HNO_3 , Br_2 or KMnO_4 . Heated in a dry tube it gave an oily acid distillate. It crystallized from water in the form of a hydrate containing 13.24% H_2O . $\text{C}_4\text{H}_6\text{O}_4 \cdot \text{H}_2\text{O}$ requires 13.22% H_2O . (Analysis of anhydrous material from toluene (Weiler). Found: C, 40.9; H, 5.00%. $\text{C}_4\text{H}_6\text{O}_4$ requires C, 40.8; H, 5.08%. Neutralization equivalent, 61. Theoretical for dibasic acid, 59.)

0.5 g. of the acid was heated in a tube and the distillate collected in an aqueous suspension of Ag_2O . The silver oxide suspension was washed and excess Ag_2O removed by filtration at 100°. The filtrate on cooling gave crystals of a silver salt which were collected and dried. A weighed amount of this was ignited and gave 59.2% Ag. The theoretical value for silver propionate is 59.3% Ag.

The acid isolated has all the properties of methylmalonic acid, the m.p. of which is stated in the literature to be between 120° and 130° according to different authors. The formation of a monohydrate by this acid does not appear to have been previously described.

More rats were fed on exactly the same diet as previously described except that no anthracene was present. The urine was collected, acidified, filtered and extracted with ether for 24 hours. The ethereal extract from 4 litres urine was boiled with charcoal, filtered and evaporated to 20 ml. The crystals which separated on keeping the residue in a desiccator were filtered off and dried; yield 0.4 g. Part of the crude material was crystallized from hot toluene and then melted at 124–126° and gave propionic acid on heating to 150°.

It was found impossible to isolate methylmalonic acid from rabbit urine or from human urine. In one experiment the combined ethereal extract of 34 litres of male human urine was treated in a manner similar to that described above.

The precursor of the methylmalonic acid is as yet unknown, but it could conceivably arise from thymine, the side chain of cholesterol, carotenoids or the methylpyrrole of haemoglobin and other pigments. Of these, however, the simplest precursor is thymine which on oxidation and hydrolysis would give urea

and methylmalonic acid. In the breakdown of thymonucleic acid, if all the purines were converted into uric acid and all the thymine into methylmalonic acid, there should be one molecule of the latter to two molecules of uric acid.

Szent Györgyi has shown that sodium malonate causes inhibition of respiration and of acetoacetate breakdown in liver slices, but we have found that sodium methylmalonate does not have this effect.

SUMMARY.

Methylmalonic acid has been isolated from rat urine where it may occur to the extent of 0.5 %.

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REFERENCE.

Boyland and Levi (1935). *Biochem. J.* **29**, 2679.