Labelling of urinary uric acid and allantoin

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In previous experiments (1) we examined the liver content of uric acid and allantoin purifying them for the first time. This was achieved by TCA extraction of the tissue, hydrolysis with IN HgSO₄ of purine bases from purine nucleotides, precipitation of purine bases, uric acid and allantoin with Hg-Acetate according to the procedure of Korn (2). The precipitated allantoin and uric acid were then dissolved in H₂O, and after elimination of Hg with Hydrogen Sulfide, further separated and purified by cation and anion exchange chromatography. The resulting compounds were absolutely pure and could be tested for specific radioactivity, when rats had previously been treated with a single dose of ¹⁴C-formate metabolic tracer. A very interesting result was that the specific radioactivity of allantoin was higher than that of uric acid shortly (15'-30'-60') after treatment, while the radioactivity of both compounds was identical after longer time intervals (154') (3). Several problems which cannot be explained by current knowledge were raised by this result, since allantoin is derived solely from uric acid and the pools of the two compounds are very similar. Since both uric acid and allantoin are formed in the liver and other organs, we must remember that liver allantoin and uric acid represent a mixed situation, and this could at least partially explain the observed result. In order to ascertain if the observed result was specific to the liver, we evaluated the radioactivity pattern of uric acid and allantoin, in the perfused rat liver, and in urine (representative of purine catabolism of the whole body) after ¹⁴C-formate load.

The perfused rat liver was prepared as previously described (4) using Krebs-Henseleit buffer, without recirculation of the perfusate (open system) at 37°C for 30'. During the following 15 min. ¹⁴C-formate (3µCi/100 g b.w.) was added to 500 ml buffer. Perfusate flow was about 4 ml/min per g wet wt. Lactate dehydrogenase activity was measured in the effluent perfusate (5).

Uric acid and allantoin were prepared from the liver, as in the case of the living animal (1).

For urinary uric acid and allantoin, rats were kept in metabolic cages, with food intake and water ad libitum. Urine was collected during different periods (8h, 24h, 48h) after intraperitoneal ¹⁴C-formate administration. Uric acid and allantoin were purified from the urine by a previous reported method (1) and were analyzed for radioactivity.

The results are reported in Tables 1 and 2.

Table 1 shows that the specific radioactivity of allantoin obtained from perfused rat liver is always higher than that of uric acid at the time (15') chosen for our experiment in vivo.

References