STUDIES ON THE METABOLISM OF RADIOACTIVE NICOTINIC ACID AND NICOTINAMIDE IN MICE*

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(Received for publication, May 21, 1948)

The use of isotopes as tracers, both stable and radioactive, has become a well established technique for the study of intermediate metabolism of many compounds of biological importance. Recently the synthesis of radioactive nicotinic acid and nicotinamide (with C\textsuperscript{14} in the carboxyl group) has been reported by Murray, Foreman, and Langham (1).

In this paper the gross metabolism of radioactive nicotinic acid and nicotinamide in mice is discussed. These results were obtained by determining the amount of radioactivity appearing in the exhaled air, urine, feces, and tissues as a function of time, following their administration by intraperitoneal injection.

EXPERIMENTAL

The apparatus which was used is shown in Fig. 1. Three mice were put into each of several cages, \( G \), on glass screen, \( K \); air (dried and freed of CO\textsubscript{2} by passage through drierite and ascarite) was drawn into the system through \( I \) at a rate of approximately 500 ml. per minute as determined by a flow meter. The exhaled CO\textsubscript{2} was collected in a train of three towers containing 20 per cent CO\textsubscript{2}-free NaOH. Urine was collected in \( A \) under toluene. Feces were collected in \( B \). In order to minimize the contamination of urine and feces with food, \( D \) was inserted to trap most of the powdered food particles. A single stick of mouse food \( \frac{1}{2} \times 4 \) inches was inserted into \( E \) on elevation \( F \), which allowed animals to eat but prevented them from gnawing off any large particles and bringing them into the cage. Water was supplied through \( H \). Funnel \( C \) gave good separation of feces and urine, each relatively uncontaminated with food.

On Day 1 of the experiment 0.7 mg. of radionicotinic acid (50,000 counts per second) was injected intraperitoneally into each of nine mice (Strain CF-1) which were then immediately put into metabolism cages (Fig. 1).

* This document is based on work performed under contract No. W-7405-eng-36 for the Atomic Energy Project, and the information covered therein will appear in Division V of the National nuclear energy series as part of the contribution of the Los Alamos Laboratory.

Presented at the 113th meeting of the American Chemical Society.
A second set of nine animals was injected with 0.7 mg. each (50,000 counts per second) of radionicotinamide and similarly treated. The $\beta$ radiation from these injections was approximately 1 roentgen equivalent per day for the 1st day and much less on succeeding days because of the high excretion rate. Urine, feces, and CO$_2$ were collected at various time intervals and $^{14}$C activity determined. All measurements of radioactivity were made with the assembly described by Dauben et al. (2).

![Diagram of all glass metabolism cage for collecting urine, feces, and CO$_2$](http://www.jbc.org/)

The Geiger-Müller tube used had a thin mica window, 5.4 cm. in diameter and 1.7 mg. per sq. cm. thick. The tube was filled to atmospheric pressure with helium saturated with alcohol at 3°C.

Urine samples were collected, diluted to 25 ml., and an aliquot plated directly on oxidized copper disks. The measured activity was corrected for mass absorption with calibration curves developed by preparing plates of increasing amounts of biological fluids containing a constant amount of a water solution of radioactive nicotinic acid.

Exhaled carbon dioxide was collected in 20 per cent NaOH and converted to BaCO₃. The BaCO₃ was precipitated onto oxidized copper disks, counted, and the results corrected for absorption by the method of Yankwich et al. (3).

The feces were dried in an oven at 96°, pulverized, and an aliquot extracted with water in a micro Soxhlet apparatus. More than 95 per cent of the C¹⁴ activity was removed in this manner. An aliquot of the water solution was plated directly and counted.

The data presented for urine, feces, and CO₂ are an average for nine mice. A second series of mice was injected with similar doses of radionicotinic acid and radionicotinamide. The animals were sacrificed at various time intervals for purposes of tissue analysis. All tissues except blood were dried in an oven at 96°; they were then burned by standard procedures and the CO₂ formed in the combustion was absorbed in 10 per cent CO₂-free NaOH, precipitated as BaCO₃, and plated onto copper disks. Radioactivity was determined as above.

Blood samples were heparinized and centrifuged. The plasma was separated from the cells and the cells laked with distilled water. Radioactivity in plasma and laked cells was determined by plating the biological fluid directly on copper disks. Corrections for absorption were made according to specially developed absorption curves.

**DISCUSSION**

Fig. 2 shows the excretion of radioactivity in the urine and CO₂ for nicotinic acid and nicotinamide. Each point represents an average value for
the nine animals studied in each group. On Day 1 there is a large excretion of radioactivity in both urine and CO₂. An initial injection of 0.7 mg. of nicotinic acid, which exceeds the normal requirement, results in a large excretion of radioactivity in the first 24 hours. The decrease in the slope of the urine curves which occurs after 24 hours is presumably due to the incorporation of the nicotinic acid and nicotinamide into the metabolic pool of the animal, whereas the initial rapid excretion may be unmetabolized injected material. On the other hand, the rapid initial excretion of CO₂ represents normal metabolism or detoxification. After the first 48 hours, when the nicotinic acid and nicotinamide are presumably in the form of coenzyme in the various tissues, a comparison of the ratio of radioactivity found in the CO₂ and urine would indicate that from 15 to 20 per cent of the

fixed nicotinic acid or nicotinamide is eliminated as CO₂. If one assumes that the radioactive CO₂ found in the first 48 hours is due only to the normal metabolic processes, it can be concluded that the total amount of fixed nicotinic acid or nicotinamide is approximately 40 per cent of the injected dose.

It is to be noted that the urine curves are parallel, as are the CO₂ curves, indicating that nicotinic acid and nicotinamide enter into the same metabolic system. This is confirmed by the results obtained on individual tissue analysis.

Work on the identification of the various metabolic products in the urine is in progress.

Fig. 3 shows the radioactivity obtained by extracting the feces. The data are plotted as a bar graph to emphasize the irregularities in excretion. Because of the low activity in the feces and the likelihood of contamination
from the much more active urine, it is felt that little significance can be attached to these data. If any fecal excretion of metabolites occurs, it is quite low.

The activity in the various tissues resulting from uptake of the injected...
nicotinic acid and nicotinamide was determined at increasing time intervals by sacrificing the animals and preparing plates for counting as described above. Fig. 4, a through h, shows these data on a semilog plot.

The results show that the uptake of radioactive nicotinic acid or nico-
tinamide varies with the different organs and is highest in kidney and lowest in erythrocytes. No radioactivity was found in the plasma after the first 24 hours. The excretion half times vary also with the different organs and are about 4 days in liver, kidney, and spleen, 5 days in cardiac muscle and
erythrocytes, and 8 days in brain, sternum, and skeletal muscle. Presumably the major portion of the radioactivity determined was in the form of coenzyme, as it has been shown repeatedly that nicotinic acid and niacinamide administered in vivo result in an increase in tissue coenzyme.
SUMMARY

1. Gross metabolism studies on nicotinic acid and nicotinamide with use of compounds tagged with C\textsuperscript{14} in the carboxyl group are presented.

2. It is shown that of the radioactivity fixed in the tissues about 15 per cent of the C\textsuperscript{14} in the carboxyl group appears as exhaled CO\textsubscript{2}. Presumably this occurs as a result of decarboxylation, but there may also be ring rupture followed by decarboxylation. In any case, the metabolites which have been isolated to date have all contained a carboxyl group in the 3 position of the pyridine ring. The fact that nicotinic acid and nicotinamide are decarboxylated should stimulate the search for additional metabolic products.

3. The gross metabolism of nicotinic acid and nicotinamide is identical in the mouse.

BIBLIOGRAPHY

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