Distribution of Nitrogen-13 from Labeled Nitrate ($^{13}NO_3^-$) in Humans and Rats

Abstract. *The body distribution of gavaged or intravenously administered nitrate labeled with nitrogen-13 was studied in humans and rats with the following results: (i) the labeled compound is not quickly absorbed from the stomach; (ii) the concentration of the label increases inside the lower intestinal tract (cecum and large intestine) when ingested or intravenously injected; and (iii) humans and rats have the capacity to store a portion of the label in their bodies. These observations indicate that depletion of body stores, the passage of nitrate down the gut, or the secretion of nitrate into the intestinal lumen may be a better explanation of the urinary, ileal, and fecal concentrations of nitrate and nitrite recently measured in humans than a bacterial nitrification reaction in the intestines, as suggested by Tannenbaum* et al. *(12).*

Nitrate (NO_3^-) and nitrite (NO_2^-) are widespread in man's environment; for example, vegetables and drinking water contain NO_3^-, and processed meats and saliva contain NO_2^-. However, relatively little is known about the distribution and metabolism of these ions, which are now recognized as components in the formation of carcinogenic *N*-nitroso compounds. Research into the pharmacology and biochemistry of NO_3^- and NO_2^- has undoubtedly been retarded by the difficulty of chemical analysis in most tissue samples (*1*) and the nonexistence of a reasonably long-lived radioisotope of nitrogen. However, ^{13}N (half-life, 10.0 minutes), produced in 1934 (*2*), is available from nuclear accelerators and is finding increasing use in nuclear medicine (*3*). Experiments of up to 2 hours duration can be carried out with this isotope, which decays by positron emission with resulting 0.511-MeV radiation. Using this isotope in the form of $^{13}NO_3^-$, we have studied the distribution and the rates of change of ^{13}N concentrations in humans and rats.

We produced 20 to 30 mCi of ^{13}N as $^{13}NO_3^-$ by bombarding a recirculating, aerated water target for 20 minutes with a 3-μA beam of 11-MeV protons from the University of Wisconsin's tandem Van de Graaff accelerator (*4*). Other workers (*5*) have found $^{13}NO_3^-$ produced in this way with a cyclotron to be contaminated with $^{13}NH_4^+$ and $^{13}NO_2^-$. Such is not the case in our apparatus. For the determination of $^{13}NH_4^+$, 1 mmole of NH_4Cl was added to a sample of irradiated water and then excess NaOBr was added. The resulting 0.5 mmole of N_2 contained less than 0.1 percent of the radioactivity. For the determination of $^{13}NO_2^-$, 1 mmole of $NaNO_2$ was added to irradiated water and then excess NaI and HCl were added. The evolved NO (collected as NO_2 after reaction with O_2) contained less than 0.1 percent of the radioactivity. The 30 mCi of $^{13}NO_3^-$ thus produced corresponds to about 10^{12} atoms (that is, about 1 pmole) of ^{13}N. This is a "perfect" tracer in that it is almost carrier-free and can be detected only by its radiation. By contrast, when stable ^{15}N is used, a large amount of the element may perturb the system being studied.

Experiments with two human volunteers (authors) were carried out in which the distribution of radioactivity was followed with a computer-linked Anger camera (*6*) after $^{13}NO_3^-$ had been taken orally (both subjects) or injected intravenously (one subject). The data obtained in this way are not fully quantitative (*7*). The distribution of ^{13}N in the body is summarized in Table 1. One subject received oral $^{13}NO_3^-$ about an hour after a large meal. The half-time for movement of the label out of the stomach was about 30 minutes. The radioactivity in the pylorus however, remained almost constant; this finding strongly suggests that NO_3^- is not rapidly transported from the stomach into the blood but rather exits into the small intestine. The half-time for movement of the label out of the stomach for the other subject was less than 10 minutes; he was given $^{13}NO_3^-$ about 10 hours after eating (Table 1).

After intravenous administration of $^{13}NO_3^-$, there was rapid distribution of the label; activity in the heart region (data not shown in Table 1) reached a maximum of about 3 percent of the injected ^{13}N at 2 minutes as the label passed through the heart for the first time and then fell rapidly in the next 2 minutes as the label became evenly distributed in the blood. Thereafter, the amount of radioactivity in the heart region fell more slowly over the next 26 minutes to about 2 percent of the injected ^{13}N as the label moved into other compartments. Furthermore, the radioactivity accumulated almost linearly with time (up to 1.5 percent of the total ^{13}N given) in an approximately fist-sized region of the abdomen (data not shown in Table 1). This was probably due to the swallowing of salivary $^{13}NO_3^-$. The remainder of the injected $^{13}NO_3^-$ was fairly evenly distributed throughout the body since less than 5 percent appeared in the urine or salivary glands in 40 minutes.

In order to study the pharmacokinetics of $^{13}NO_3^-$ in greater detail than is possible in humans, we gavaged or intravenously injected $^{13}NO_3^-$ into conventional-flora (CV) rats. The body distribution of ^{13}N in rats given $^{13}NO_3^-$ orally with and without pyloric ligation is shown in Table 2. Since the group with pyloric ligation retained nearly all the radioactivity in the stomach after 40 to 50 minutes (compared to 21 percent in the rats without pyloric ligation), it appears that $^{13}NO_3^-$ leaves the rat stomach predominantly through the pyloric valve, an observation which agrees with our conclusion for humans. The low counts noted in the duodenum of nonligated (pyloric), gavaged rats suggests that either $^{13}NO_3^-$ is rapidly absorbed from, or moves quickly through, this upper region of the intestinal tract. In contrast, the high counts at 45 to 60 minutes in the jejunum and ileum of gavaged rats suggest one of the following: (i) that $^{13}NO_3^-$ (as such) is not totally absorbed from these segments of the intestinal tract; (ii) that the chemical form of ^{13}N has changed and this has influenced its absorption; or (iii) that ^{13}N is being secreted into the lower small intestine from the bloodstream. Interestingly, most of the orally gavaged radioactivity in the nonligated rats was found in the carcass (that is, the rat body minus the organs specified in Table 2). This carcass ^{13}N was more than could be accounted for as blood-bound activity.

To study the fate of bloodstream NO_3^-, we injected $^{13}NO_3^-$ intravenously into unstarved, unligated CV rats (Table 2). Except for the stomach, no marked accumulation of ^{13}N was seen in any organ and the label was mainly in the eviscerated carcass at 50 minutes. Counts in the bladder (and kidney) of these rats increased (Table 2) to a level comparable to that of the human subject intravenously injected with $^{13}NO_3^-$ (Table 1). The salivary glands of these five intravenously injected rats, however, accumulated activity (not shown in Table 2) to only one-tenth (that is, 0.4 percent) of that seen in the human mouth (Table 1). Since the percentage distribution of ^{13}N along most the gastrointestinal tract of nonligated, gavaged rats (Table 2) was greater than that of nonligated rats injected intravenously (Table 2) with $^{13}NO_3^-$, ^{13}N probably traveled to the lower small intestine (ileum) by movement out of the stomach with digested food. However, as seen in the

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group of rats with pyloric ligation 30 minutes after injection of $^{13}NO_3^-$, the intestinal tract from the duodenum to the large intestine contained essentially the same amount of ^{13}N whether or not the pylorus was ligated (Table 2). This result indicated that $^{13}NO_3^-$ could enter the intestines from the blood either as part of the biliary or pancreatic secretions or by direct secretion from the bloodstream.

To help clarify whether ^{13}N could be secreted from the bloodstream into the intestine, we carried out ileocecal ligation on a group of rats before intravenous injection of $^{13}NO_3^-$ (Table 2). The proportion of label in the lumen of the lower intestinal tract increased with time (data not shown); this result suggests that $^{13}NO_3^-$ was secreted directly into the intestines from the blood. In addition, the total amount of label in the ceca of rats intravenously injected after ileocecal ligation was greater than in nonligated intravenously injected controls (Table 2). This finding indicates that certain bowel alterations (that is, resection or strangulation) may enhance the passage of NO_3^- into the intestinal contents.

These ^{13}N data suggest the following. (i) The body may act as a temporary reservoir of ^{13}N. A significant amount of this label would probably be in the form of NO_3^-, since it does circulate as such in the bloodstream of man and accounts for urinary and salivary NO_3^- concentrations (*8*). (ii) The ^{13}N does pass beyond the upper small intestine (duodenum and jejunum) when ingested. (iii) Biliary, pancreatic, or intestinal secretions may contribute to the pool of ^{13}N present in the lower small and large intestines. (iv) Bowel alterations may enhance the influx of NO_3^- into the bowel contents.

Research by others tends to support our observations. For example, Wang *et al.* (*9*) incorporated $^{15}NO_3^-$ and $^{15}NO_2^-$ as 2.0 and 1.6 percent, respectively, of the food given to rats (Holtzman strain) for 14 hours. Seventy-two hours after completion of this labeled (^{15}N) meal, 10 to 11 percent of the ^{15}N of the NO_3^- and NO_2^- remained in the rat carcasses. Early studies with humans fed saltpeter (KNO_3) also showed that an increase or decrease in the amount of NO_3^- ingested did not result in a concomitant increase or decrease of urinary NO_3^- (*10*). In both these instances, it appears as though the carcass was able to store the label (^{15}N) or the ion (NO_3^-) and could thus act as a temporary reserve of NO_3^-.

It is important to determine by further experiments the extent to which ^{13}N radioactivity is still $^{13}NO_3^-$ when measured. We know of no mammalian enzymes which can convert NO_3^- into other compounds. However, bacteria in the mouth and intestines can reduce NO_3^- to NO_2^-, which can react with amines and amides to form carcinogenic *N*-nitroso compounds. The NO_2^- can also react with hemoglobin in blood and can undergo further reduction in the gut or reoxidation to NO_3^- in the stomach. For $^{13}NO_3^-$, administered intravenously, we can be fairly confident that the bulk of the label, over the time course of this experiment, would stay as $^{13}NO_3^-$. The same cannot be said for $^{13}NO_3^-$ administered orally, since this entered the circulation from the intestines where reduction or other microbial or chemical transformations might have occurred. However, radioactivity in the salivary glands in human studies (Table 1) strongly suggests that much of the label is still $^{13}NO_3^-$ (*11*).

Tannenbaum *et al.* (*12*) have recently suggested that human intestinal flora can produce NO_3^- and NO_2^-, presumably from NH_4^+, by a bacterial nitrification reaction in the intestines. They showed that human volunteers who were maintained on "protein-free" diets continued

Table 1. Percentage distribution of ^{13}N from $^{13}NO_3^-$ administered to human volunteers by oral (two subjects) or intravenous (one subject) routes; N.D., not determined.

Body site	Intravenous $^{13}NO_3^-$		Oral $^{13}NO_3^-$		
	Time (minutes) after injection	% of counts*	Time (minutes after drinking)	Full stomach (% of counts)	Empty stomach (% of counts)
Bladder	31 to 36	3	37 to 41	2	N.D.
Mouth and salivary glands	37 to 41	4	31 to 36	3	N.D.
Stomach	N.D.	N.D.	10	100	87
Stomach	N.D.	N.D.	20	88	27
Stomach	N.D.	N.D.	30	72	9

*Counts are corrected for the 10-minute half-life of ^{13}N but not for different attenuation in different areas of the body. Percentages are related to the total counts in the stomach when $^{13}NO_3^-$ was given orally (*7*). The subject lay face up with the camera (Nuclear Chicago Pho/Gamma HP, with high-energy collimator and interfaced with a PDP 11-40 computer) over the stomach. Frames were taken at the rate of one per minute for 30 minutes. One 5-minute frame was then taken for the head and shoulders and for the bladder.

Table 2. Distribution of ^{13}N in rats with and without a gastrointestinal ligation (pyloric or ileocecal) 20 to 60 minutes after $^{13}NO_3^-$ was gavaged (oral) or intravenously injected (cardiac puncture). Sprague-Dawley CV rats (approximately 250 g each) were starved for 18 hours before oral administration; rats injected intravenously were not starved. Rats were etherized and rapidly dissected 15 to 60 minutes after gavaging with $^{13}NO_3^-$ (2 ml containing approximately 1 mCi of $^{13}NO_3^-$). The organ radioactivity was counted with a NaI (Tl) crystal detector for two 10-second periods. Counting geometry was rigorously maintained for all tissues except for the initial whole body and the final eviscerated carcass, which were placed 15 cm above the crystal. Corrections were applied to allow comparison of these values with those obtained for the other organs, and all counts were decay-corrected to the start of dissection. Values are the mean ± the standard error of the mean, if applicable. Sham-operated controls for the oral administration showed distributions similar to those of the nonligated rats.

Type of ligation (No. rats used)	Time (minutes)	Percentage distribution of ^{13}N						
		Stomach	Duodenum	Jejunum	Ileum	Lower intestine*	Kidneys and bladder	Carcass
				Oral				
None (9)	45 to 60	21.4 ± 17.1	3.0 ± 2.8	13.0 ± 7.5	4.1 ± 0.5	0.7 ± 0.3	5.1 ± 4.8	47.3 ± 8.7
Pyloric (3)	40 to 50	94.6 ± 3.6	0.2 ± 0.1	0.3 ± 0.4	0.2 ± 0.2	0.2 ± 0.1	0.1 ± 0.2 †	4.0 ± 8.7
				Intravenous				
None (5)	20 to 50	6.8 ± 2.1	1.2 ± 0.5	2.2 ± 0.5	0.8 ± 0.3	1.6 ± 0.5	3.1 ± 0.5	74.8 ± 6.3
Pyloric (1)	30	5.6	1.1	2.1	1.4	1.9	5.2	78.8
Ileocecal (3)	20 to 45	6.1 ± 3.1	1.4 ± 0.3	1.4 ± 0.1	1.2 ± 0.2	3.0 ± 2.1	1.3 ± 0.9	74.2 ± 5.9

*The cecum and large intestine. †Bladder only.

to excrete NO_3^- in the urine for at least 20 days. The total excess urinary NO_3^- was 1 to 2.5 g. However, the assumption of a nitrification process in the gut cannot be interpreted properly unless values for the total body content of NO_3^- and NO_2^- are known. We have been unable to find such values in the literature. Radomski *et al.* (*1*) have also commented on the lack of available information about the body disposition of NO_3^-. They point out the difficulties associated with assaying NO_3^- and in excluding this ion from food and drinking water. However, a rough estimate of 1 g for the total body content of NO_3^- can be made from the data in Table 1 if the following assumptions are made: (i) intravenous $^{13}NO_3^-$ equilibrates with body NO_3^- in 40 minutes; (ii) the bladder contained 250 ml of urine; and (iii) the concentration of NO_3^- in urine was 100 mg/liter reported by Radomski *et al.* (*1*). Since this is the same order of magnitude as the excess urinary excretion reported by Tannenbaum *et al.* (*12*) and since $^{13}NO_3^-$ was distributed very evenly, it seems possible that their results could be explained (at least in part) as due to the slow washout of NO_3^- from the body. Furthermore, the ^{13}N data in rats may help explain the intestinal values of NO_3^- and NO_2^- reported by Tannenbaum *et al* (*12*). Although a major percentage of the ^{13}N appears to be absorbed from the upper intestinal tract, clearly it is not entirely removed as suggested by Hill and Hawksworth (*13*). Therefore, direct passage of NO_3^- and NO_2^- down the gut, which previously was though not to occur, in conjunction with the secretion of bloodstream (or biliary and pancreatic) NO_3^- into the intestinal lumen (which an intestinal alteration, that is, colectomy, may enhance) could account for the presence of NO_3^- and NO_2^- in the ileostomy and fecal samples as reported (*12*).

Further work should be done to delineate whether these intestinal and urinary concentrations of NO_3^- and NO_2^- are the result of dietary intake, slow release of body stores, or bacterial nitrification in the intestinal tract (*12*). Since the last of these explanations would represent an unavoidable exposure to these ions and hence the possibility for a "complete" endogenous formation of carcinogenic *N*-nitroso compounds, its importance is obvious.

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6. An Anger camera consists of a single crystal of NaI (doped with thallium) typically 1 cm thick and 30 cm in diameter. A lead collimator in front of the crystal improves resolution at the expense of sensitivity. Signals from 20 or more photomultipliers behind the crystal are processed to give x and y coordinates of each γ-ray that produces a scintillation in the crystal. The computer synthesizes a 64 by 64 matrix picture (a "frame") from these coordinates, and the spatial resolution is about 1 cm. Nuclear medicine imaging and other procedures are described in *A Textbook of Nuclear Medicine Technology*, P. J. Early, M. A. Razzak, B. Sodee, Eds. (Mosby, St. Louis, 1975).
7. The results in Table 1 can be approximately related to one another because the total counts were measured in an internal organ (the stomach) where conditions for the attenuation of radiation would be similar to those for salivary glands and the bladder. Furthermore, the attenuation of 0.511-MeV radiation by tissue is not great.
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11. In similar intravenous studies with humans using $^{13}NH_4^+$, we found that the salivary glands do not "concentrate" ^{13}N as they do when $^{13}NO_3^-$ is injected.
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14. S.J.G. thanks the Wellcome Trust for a travel grant. We thank the University of Wisconsin physics staff for generous allocation of accelerator time [Energy Research and Development Administration grant EY-76-C-02-0007-00 (Task A), NCI 5PO1CA 19278-03]; Dr. R. J. Nickels for supplying us with $^{13}NO_3^-$; G. D. Peterson, C. Kan, B. Johnson, and J. Brown for help with the animal experiments; and D. Brackett for her patience in manuscript preparation.

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Influence of Cartilage Geometry on the Pressure Distribution in the Human Hip Joint

Abstract. *To elucidate the role of mechanical factors in the etiology of osteoarthritis, the detailed geometry of the weight-bearing cartilage layer over the human hip socket is compared with the corresponding pressure distribution. The shape of the pressure distribution is strongly correlated with the shape of the cartilage compression distribution.*

Articular cartilage is a remarkable bearing material that can provide a lifetime of trouble-free movement of synovial joints. Often, however, signs of cartilage deterioration are seen in the joints of middle-aged persons, and the incidence and severity of degeneration generally increase with age (*1*). Despite many nutritional, biological, and biochemical studies (*2*), the cause of osteoarthritis is still unknown. More recently, researchers have emphasized the importance of understanding the frictional, wear, and mechanical behavior of articular cartilage—that is, lubrication mechanisms, anisotropic, poroelastic, and viscoelastic properties, and fatigue strength (*3*). To understand the mechanical environment in which articular cartilage must exist in vivo, we developed a new, more accurate description of the global shape and thickness of acetabular cartilage by using ultrasonics and compared this geometry with the corresponding surface pressure distribution on loaded acetabula from cadavers.

The pressure distribution is of particular importance for understanding the par-

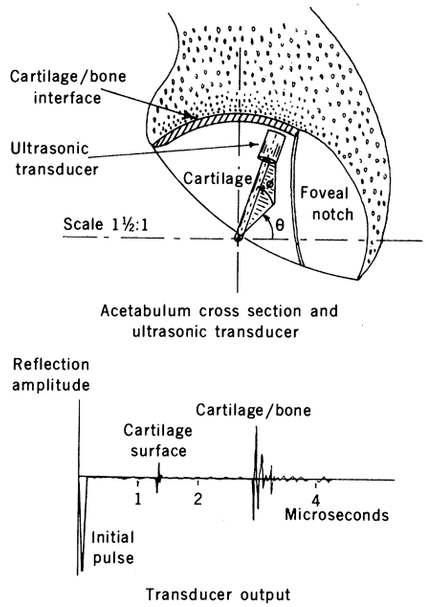

Fig. 1. Schematic representation of the ultrasonic transducer scanning technique used to measure hip joint geometry.

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