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A Research Report

Final Report

Total Body Nitrogen Analysis

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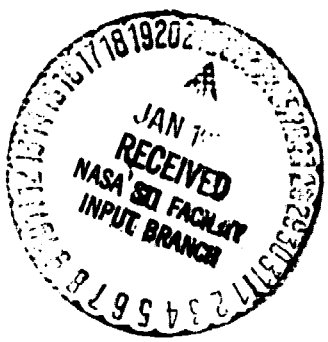

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TOTAL BODY NITROGEN ANALYSIS

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ABSTRACT

Studies of two potential in vivo neutron activation methods for determining total and partial body nitrogen in animals and humans are described. A method using the ^{11}CO in the expired air as a measure of nitrogen content was found to be adequate for small animals such as rats, but inadequate for human measurements due to a slow excretion rate. Studies on the method of measuring the induced ^{13}N in the body show that with further development this method should be adequate for measuring muscle mass changes occurring in animals or humans during space flight.

INTRODUCTION

The purpose of this work is to develop a method for determining changes in muscle mass which may occur in astronauts and animals during space flight. Skylab data have indicated a change in regional and total muscle mass in space flight crew members in spite of exercise and dietary regimes designed to prevent these changes. Quantitative and qualitative data are necessary to explain these changes and present methods are not adequate to obtain this data. This work is directed towards the development of accurate methods for muscle mass measurements.

The relationship between muscle mass and protein and between the protein and nitrogen content of the body indicate that the muscle mass is directly related to nitrogen content. Muscle mass is also directly related to the potassium content of the body which can be measured by determining the naturally occurring ^{40}K by whole body counting; however, the precision of this measurement is limited to ± 4 percent for the total body and the precision is much worse when measuring specific sections of the body such as an arm or leg. With in vivo neutron activation it appears feasible to measure changes in nitrogen content which exceed 2 percent in either the total body or a body part.

There are three possible metnods for measuring total body or partial body nitrogen in vivo using neutron activation. One method which has been used for mice[1] and to a very limited extent with humans[2] uses fast neutrons to produce the reaction ^{14}N (n, 2n) ^{13}N. The ^{13}N has a 9.96 minute half-life and emits two 0.51 MeV annihilation photons per disintegration. Most of these photons escape the body and can be measured with a whole body counter.

Another method measures the prompt 10.8 MeV gamma emitted when ^{14}N captures a thermal neutron.[3] The irradiation and counting are done during alternating short periods while the patient remains in a fixed position. The neutrons from a cyclotron are pulsed on for a period of 10 microseconds during which the counting is stopped. After the neutrons are turned off, the counting is performed during the next few hundred microseconds as the

neutrons are thermalized and captured within the body. This sequence of neutron pulses followed by counting periods is continued for about 12 minutes. The main purpose of using pulsed neutrons is to eliminate the high gamma ray background produced during the neutron production in the lithium target. This method appears to be the least practical of the three methods.

In a third method which has been used with animals,[4] the ^{11}C exhaled in the expired air which is produced by the reaction $^{14}N\ (p,\alpha)\ ^{11}C$ is measured. The ^{11}C has a 20.4 minute half-life and is easily recovered from the expired air and measured.

The main effort of this work was devoted to the ^{13}N and ^{11}C methods to determine which of the two would be the best for use in the space program.

PART I

INVESTIGATION OF THE USE OF ^{11}C IN EXPIRED AIR AS A MEASURE OF TOTAL BODY OR PARTIAL BODY NITROGEN AFTER NEUTRON IRRADIATION

In the first section of work done under this contract[4] it was discovered that relatively large amounts of ^{11}C occur in the expired air of rats exposed to neutron irradiation. Further studies showed that the ^{11}C is produced only from a (p,α) reaction on the stable ^{14}N in the body. The energetic protons for the reaction result from fast neutrons interaction with the hydrogen in the body. The ^{11}C leaves the body as both ^{11}CO and $^{11}CO_2$ and the excretion rate is exponential. The excretion rate of the $^{11}CO_2$ can be doubled by exercise or cold environment. The excretion rate of the ^{11}CO is also effected similarly by exercise or cold environment but more important is the fact that the rate of ^{11}CO excretion is increased by a factor of 5 when the animal breathes pure oxygen rather than normal air. This rapid excretion rate allows the ^{11}CO to be removed from the body and counted before the 20.4 minute half-life ^{11}C decays away.

Interferences in the ^{11}C Method

A study was made of all the radioactive gases produced in expired air after neutron irradiation. These studies were done with rats irradiated with 14 MeV neutrons. The gases detected were ^{37}A, ^{41}Ar, $^{11}CO_2$, ^{11}CO, ^{13}NO, $^{13}N_2O$, $^{13}N_2$, and possibly some $^{11}CH_4$. Since soda lime was used to trap the $^{11}CO_2$, the ^{37}Ar, ^{41}Ar, and $^{13}N_2$ would pass through the soda lime and would not produce any interference. The ^{13}NO, $^{13}N_2O$ being acid gases would be collected on the soda lime trap along with the $^{11}CO_2$. The ^{11}CO passes through the soda lime trap and is then oxidized to $^{11}CO_2$ on hot CuO and is then collected on a second soda lime trap. If a trace of $^{11}CH_4$ does exist, it would pass through the first soda lime trap, be oxidized on the hot CuO and converted to $^{11}CO_2$, but since the $^{11}CH_4$ is also produced from ^{14}N in the body this would not provide any interference to the method.

Since the $^{11}CO_2$ fraction of expired air is released too slowly to be useful, the absorption of the nitrogen oxides on this trap does not present a problem and in fact removes them so that they do not interfere with the ^{11}CO measurement.

A review of all possible nuclear reactions with the body elements failed to turn up any other mechanism by which ^{11}C can be produced in the body with 14 MeV neutron irradiation. Stable ^{14}N appears to be the only source of the ^{11}C.

Comparison of the Yield of ^{11}C to ^{13}N in the Body

The ^{11}C produced in the body is only a few percent of the amount of ^{13}N produced in the body during the same neutron irradiation. When an aqueous solution of NH_4NO_3 was irradiated with 14 MeV neutrons, the measurable activity of the ^{11}C flushed from the solution was only about 3 percent of that from the ^{13}N which was produced and remained in the solution. Approximately the same results were obtained from an irradiated rat. When the rat results were extrapolated to the size of man, a value of 56 mrad neutron dose would give about 10,000 usable counts from $^{11}CO_2$ and 112,000 possible counts of ^{13}N from the body if a very efficient whole body counter is used. The count rate of the ^{13}N is very dependent on the whole body counter efficiency. Although the production of ^{11}C is only a few percent of the ^{13}N production for the same amount of neutron irradiation, the fact that it is released from the body, then collected and concentrated into a small volume, allows it to be counted much more efficiently than if it were in the body. If the ^{11}CO remained in the body, it would be impossible to quantitatively measure it because of the much larger quantity of ^{13}N present which emits the same photon energy.

Precision of ^{11}CO Measurement

The ^{11}CO was collected and measured each time after three irradiations in a single day. The rat was irradiated uniformly and precisely each time by a method described in a previous report.[5] The ^{11}CO was collected and counted from three 30-minute fractions of expired air starting at the

beginning of the neutron irradiation. The rat breathed pure oxygen and the rate of ^{11}CO excretion was the same in all three measurements. The total ^{11}CO measurement after each of the three irradiations varied within $\pm 2\%$ which indicates the method has a precision equal to other in vivo neutron activation methods when done repeatedly on the same animal or person.

Excretion Rate of ^{11}CO from Humans

The studies with rats indicate that the ^{11}CO is released from the body with a half time of about 15 minutes when the rat breathed pure oxygen. This resulted in more than 99% of the ^{11}CO being released within a 90 minute period after the start of the irradiation. This excretion rate is adequate to obtain a measurement of the total ^{11}CO produced in the body before it decays away.

A study was conducted with Dr. Nelp's group at the University Hospital at the University of Washington to determine the excretion rate of ^{11}CO in expired air from a patient after neutron irradiation. The patient was being irradiated for the purpose of determining total body calcium by whole body counting. The ^{11}CO excretion was measured as a side study without interfering with the calcium measurements. The patient breathed pure oxygen during the collection of ^{11}CO. In this study the excretion rate was much slower than in the rat. More studies would be needed to more accurately determine the excretion rate but the results of this one study show that at least 50% of the ^{11}CO still remained in the body after 90 minutes. This slow excretion rate would not allow an accurate measurement of the total ^{11}CO formed in the body and therefore the ^{11}CO could not be used as a measure of total body nitrogen in humans. Although it would be interesting to determine why the tremendous difference exists between rats and man in ^{11}CO excretion we will not pursue this further at this time.

Application of ^{11}CO Measurements for Body Nitrogen Determination

The ^{11}CO method appears very feasible for use in determining total body nitrogen in small animals such as the rat. Its use in humans does not look promising because of slow excretion rate. Even if the excretion rate had

been rapid enough the ^{11}CO method would not be useful for partial body measurements unless a rather high neutron exposure is used. A radiation dose of 56 mrads would have been necessary for whole body irradiation to get at least 10,000 net counts. If only a fraction of the body is measured such as a lower leg, a dose of 500 to 1000 mrad to the leg would be required to get sufficient ^{11}CO production in that part to provide the necessary counting accuracy after it is released in the expired air.

It appears that the ^{11}CO method can only be used for total body nitrogen of small animals and that further studies on methods for total body nitrogen measurement should be done with the ^{13}N method. Part II of this report discusses some studies on this method.

PART II

INVESTIGATION OF THE METHOD FOR DETERMINING TOTAL BODY NITROGEN BY MEASURING THE ^{13}N PRODUCED IN THE BODY AFTER NEUTRON IRRADIATION

Interferences

In earlier studies, it was assumed that the only sources of error for ^{13}N measurements were the ^{13}N which is produced from oxygen in the body, and ^{30}P, which is a 2.5 minute half-life positron emitter. We were unaware of the possibility that some of the ^{13}N could be expired and released from the body. During this reporting period, we have studied in some detail the extent of all possible interference in the use of ^{13}N as a measure of total body nitrogen. Since all positron emitters emit the same annihilation 0.51 MeV photons which are usually used to measure the amount of the emitter, we looked at all possible sources of positron emitters in the body. Water solutions of each of the major body elements were irradiated individually and the resulting 0.51 MeV photon activity was multiplied by a body composition factor for that element to determine the percent of the total 0.51 MeV activity contributed by each element. The results are listed below in Table 1.

TABLE 1

RELATIVE CONTRIBUTION OF 0.51 MeV RADIATION FROM BODY ELEMENTS

Body Element	% in Body by Weight	% of the Total 0.51 MeV Activity after Neutron Irradiation	
		10 min. after Irradiation	20 min. after Irradiation
O	65	14.5	15.3
C	18	0.0	0.0
H	10	0.0	0.0
N	3	77.6	82.6
Ca	1.5	0.0	0.0
P	1.0	6.8	0.9
S	0.25	0.0	0.0
K	0.20	0.6	0.5
Cl	0.15	0.5	0.7
Na	0.15	0.0	0.0

These results shown in Table 1 confirm that O and P are still the major interferences and, if 20 minutes is allowed for the 2.5 minute ^{30}P to decay, the P interference becomes very small. The ^{13}N interference resulting from the body water should be constant and a 5-pound change in total body water in a 170 pound man would only cause a 0.4% error. The correction of the 0.51 MeV counts for O, P, K, and Cl interference should be a constant function of body weight, and the error in the final measurement due to variability in the correction factor should be much less than 1%.

In a living animal some of the ^{13}N produced in the body is released in the expired air as nitrogen oxides along with a smaller fraction released as $^{13}N_2$. The respirable ^{13}N is a small (approximately 1%) fraction of the total ^{13}N produced in the body. Most of this expired fraction is probably due to the ^{13}N formed from oxygen in the body by a (p,α) reaction which produces a "hot atom" of ^{13}N which leaves the water molecule and immediately reacts with surrounding oxygen or hydrogen atoms. If this is the case then the portion of the whole body count due to oxygen derived ^{13}N would be less than the 15% shown in Table 1. The results in Table 1 are from solutions in which the radioactive gases are not allowed to escape. It appears that a very insignificant fraction of the ^{13}N formed from body nitrogen is released in the expired air.

Correlation of Potassium, Nitrogen, and Induced ^{13}N Activity in Rats

Previous in vivo neutron activation studies have not compared the ^{13}N produced in animals by neutron irradiation with the nitrogen content of the animals determined by chemical analysis. A study was conducted on four rats ranging in weight from 350 to 712 grams. Each live rat was irradiated and counted for ^{13}N content two times in the same day. The duplicate results were within $\pm$2.5% for each rat. The rats were sacrificed and frozen the day of irradiation so that the nitrogen content would remain constant. Each rat was placed in liquid nitrogen. At this temperature the entire rat could be crushed into small pieces and the small pieces were disintegrated into a fine powder in a high speed laboratory blender which was also kept

at liquid nitrogen temperature. The powdered tissue was brought to room temperature and stirred until a homogeneous mixture was obtained.

A 250 gram fraction of each rat was counted in a low-level background counter to determine the ^{40}K content. A standard solution of the same weight and containing 7.73 grams of potassium was also counted. The potassium content of each rat was calculated from these results. Another fraction of the homogenized rat was submitted to an outside laboratory for nitrogen analysis. The results of the nitrogen and potassium content of the rats and the ^{13}N counts are tabulated in Table 2.

TABLE 2

COMPARISON OF POTASSIUM AND NITROGEN CONTENT WITH ^{13}N COUNTS AFTER NEUTRON IRRADIATION OF RATS

Rat Weight (grams)	K (grams)	N (grams)	^{13}N (relative count)
350	.79	9.3	9,217
450	1.03	11.7	12,127
562	1.25	15.1	14,491
712	1.41	18.7	15,696

There is good correlation between the results in Table 2 considering that the accuracy of each determination was probably on the order of $\pm 3\%$. Assuming that the K determination was the most accurate the ratios of N to K had a range of 5%. However, it appears that there was greater self-absorption of gamma rays in the larger rats than in the smaller ones in the ^{13}N counting and there may have been some moisture losses from the homogenized tissue during storage before the N analysis. Corrections for these effects would produce better agreement between the three measured components. Although the experiment could be repeated with more careful attention to some of the problems which occurred, the results are adequate to show that ^{13}N measurements are as good a measure of total body nitrogen as are K and N measurements.

For in vivo measurements the ^{13}N measurements will be much better since the direct measurement of N is not possible and the measurement of total body K in a live rat is very difficult.

Muscle Equivalent Material for Phantom Studies

A material is needed for use in anthropomorphic phantoms which has the same density, nitrogen content, and rigidity as muscle tissue. Water solutions of gelatin were tested for this purpose and it was found by irradiating solutions of various gelatin concentrations that a solution containing 1 part gelatin to 5 parts water gave the same ^{13}N counting rate as the same volume of beef muscle. The solution can be prepared as a liquid and poured into the phantom parts containing the skeletal members where it sets to a very firm gel which is stable for several months. Future studies will use this combination to determine the optimum parameters for measuring partial body nitrogen content changes, which may occur during space flight.

Counting Techniques for ^{13}N Measurement in Humans

Nigrogen-13 decays by positron emissions followed by the simultaneous emission of two anihilation 0.51 MeV photons in exact opposite directions. The use of large coincidence counters for use in total body and partial body counting was considered. The use of coincidence counting would eliminate the interference from the other radionuclides formed in the body except ^{30}P. A study was made of the results from a large 4π liquid scintillator whole body counter in which the upper half of the scintillator was operated in coincidence with the lower half.[6] Due to the relatively poor energy resolution of the counter the sensitivity of the counter was increased only a factor of 2 by using the coincidence mode of counting.

A 4π NaI(Tl) 4π counter would provide a much greater increase in sensitivity and would reduce the neutron radiation dose required to only a few mrad. However the cost of such a counter would be between $300,000 to $400,000 which appears to be beyond the funding ability of these studies.

In further work, whole body counting will be done using several large NaI(Tl) detectors in the normal mode inside a shielded room. The partial body counting will be done using two of these large NaI(Tl) detectors on opposite sides of the arm, leg or other part and counting in the coincidence mode In applying the coincident requirement to this assembly, only that radioactivity located directly between the two detectors will be measured and radioactivity coming from any other part of the body will be rejected.

Summary

The ^{13}N method appears to be the best in vivo method for determining changes in muscle mass during space flight. The interferences to the method are small and constant which allow accurate corrections to be made. From the experience gained from the experiments in these studies and other types of in vivo neutron activation studies it appears that a method which can detect muscle mass changes which exceed 2% in the total body or body part can be developed for use with humans and animals.

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