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FATE OF UDMH AND MMH IN RATS

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The experiments reported herein were conducted according to the "Principles of Laboratory Animal Care" established by the National Society for Medical Research.

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FOREWORD

This study was initiated by the Biomedical Laboratory of the Aerospace Medical Research Laboratories, Aerospace Medical Division, Wright-Patterson Air Force Base, Ohio. The research was performed in support of Project No. 6302, "Toxic Hazards of Propellants and Materials," Task No. 630202, "Pharmacology and Biochemistry," under Contract No. AF 33(657)-11757, with the Science Research Institute, Oregon State University, Corvallis, Oregon. Dr. C. H. Wang was the principal investigator for Oregon State University and A. A. Thomas, MD and K. C. Back, PhD were contract monitors for the Toxic Hazards Branch, Physiology Division. Research was initiated 1 June 1963 and completed 30 June 1964.

The assistance of Mr. Royal D. Barbour and Mr. Darrell Marks in the conduct of this work is greatly appreciated.

This technical report has been reviewed and is approved.

WAYNE H. McCANDLESS
Technical Director
Biomedical Laboratory

ABSTRACT

Many of the applications of hydrazines, especially as rocket propellants and in medicine, give them considerable toxicological importance. The respiratory and urinary excretion by rats of unsymmetrical dimethylhydrazine (UDMH) and monomethylhydrazine (MMH) and their metabolites has been studied by means of radiotracer techniques. At a very low dose, almost 30% of the C^{14} from i.p. administered UDMH-C^{14} appeared as respiratory $C^{14}O_2$ in 10 hours. At a convulsive dose, the conversion of UDMH-C^{14} to $C^{14}O_2$ amounted to slightly greater than 13% at the end of 20 hours. At all doses studied radioactivity appeared in the urine to the extent of at least 50% of the administered UDMH-C^{14}, at the end of two days after administration. Rats administered MMH-C^{14} by i.p. injection at 20% of a median lethal dose respired approximately 45% of the administered radioactivity in 24 hours. The respired radioactivity consisted of at least two components; 20-25% was $C^{14}O_2$, and the remainder was a C^{14} labeled volatile compound tentatively identified as methane-C^{14}. At the sub-convulsive doses, 40% of the administered radioactivity in MMH-C^{14} was excreted in urine. At a toxic dose the percentage of urinary excretion of C^{14} decreased, but net molar excretion increased slightly. The design of an animal radiorespirometric system capable of continuously monitoring C^{14} in respiratory gases from separate animals is described.

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SECTION I

INTRODUCTION

Hydrazines are of considerable toxicologic importance due to their diverse applications ranging from rocket propulsion to employment in drugs having powerful central nervous system effects (ref. 1). Several investigators have reported on the toxicologic and pharmacologic properties of simple hydrazines (ref. 2, 3, 4, 5, 6 & 7), but only a few reports have appeared concerning their metabolic fate in intact animals. The first steps in understanding the mechanism of pharmacologic action of these agents are in studies of distribution within the body, and of the ability of intact animals to metabolize and excrete these compounds or their metabolites.

The present report describes experimental findings on the metabolic fate of unsymmetrical dimethylhydrazine (UDMH) and monomethylhydrazine (MMH) obtained by means of radiotracer methods. Previously, Back et al (ref. 8) reported the absorption, distribution and excretion of UDMH in rats, rabbits, cats, dogs and monkeys by use of C^{14} tracer and colorimetric methods. An earlier report by Reed et al (ref. 6) essentially confirmed the observations of Back et al that UDMH is not preferentially concentrated in any organ or tissue. Back et al also gave evidence suggesting a metabolite of UDMH in blood from UDMH-treated animals.

Experimental findings in our laboratory revealed that UDMH-C^{14} can be converted to some extent to respiratory $C^{14}O_2$ by intact rats. The present work extends these studies on UDMH metabolism and excretion by the rat.

Little is known of the metabolic fate of MMH. The present report describes experiments on the conversion by rats of MMH-C^{14} to respiratory $C^{14}O_2$ and a non-CO_2 volatile C^{14} compound, and two unidentified urinary C^{14} compounds. The continuous assay of C^{14} labeled compounds in the respired gas was implemented by the use of a specially designed animal radiorespirometer equipped with digital data presentation. The apparatus is described in detail in the appendix.

SECTION II

MATERIALS

UDMH-C^{14} (sp.act. 1.2 mc/mmole) and MMH-C^{14} (sp.act. 0.8 mc/mmole) were synthesized by New England Nuclear Corporation, Boston, Massachusetts. The purity of UDMH-C^{14} was established by means of gas and paper chromatography. The purity of MMH-C^{14} was established by paper chromatography and by preparation and characterization of a derivative;

2-isonicotinyl, 1-methylhydrazide (ref. 9). C^{14} labeled UDMH and MMH were stored in glass ampoules, under vacuum, in 100 μc and 200 μc lots. Unlabeled UDMH and MMH were obtained from Matheson Coleman and Bell Company, East Rutherford, New Jersey; and their respective purities were established by means of gas and paper chromatography.

Prior to the experimental use of either the labeled or unlabeled hydrazines, UDMH was dissolved in distilled water and MMH was dissolved in 0.5N HCl to a concentration appropriate for the particular experiment. Handling of each of these two compounds was carried out under nitrogen atmosphere to prevent air oxidation. HCl was used as a diluent to improve the stability of MMH. Preliminary studies indicated that the presence of HCl does not alter the toxic action of MMH with respect to lethality, toxic symptoms or metabolic manifestations.

The rats used in these studies were Sprague-Dawley males, 250 grams in body weight and were obtained from Pacord Research, Inc., Portland, Oregon. The rats were maintained on Purina Laboratory Chow and water ad libitum.

SECTION III

METHODS

Administration of the Hydrazines to Intact Rats

The hydrazine compounds were injected intraperitoneally as aqueous solutions, in volumes less than 1 ml. Dosage varied according to the objective of the experiment and in no case exceeded 75% of an established median lethal dose. These are approximately 1.8 mmole/kg body weight in the case of UDMH, and 0.6 mmole/kg body weight in the case of MMH.

Metabolic Fate Studies

Urinary radioactivity derived from UDMH-C^{14} or MMH-C^{14} was assayed by means of liquid scintillation counting techniques. In a typical procedure, 10 μl of urine was transferred into a glass counting vial containing 6 ml of an ethanol-ethanolamine solution (1:2 v/v) plus 10 ml of toluene containing 0.3% terphenyl and 0.003% POPOP (1,4-bis-2-(5-phenyl-oxazole)-1 benzene). The samples so prepared were counted in a liquid scintillation spectrometer (Model 314-EX, Packard Instrument Company, LaGrange, Illinois). A sufficient number of counts were collected to insure that the relative standard deviation of the counting data was no greater than 2%.

The radioactivity in the respiratory gases was monitored continuously by means of an animal radiorespirometer constructed for this purpose. The

design of the radiorespirometer is described in detail in the appendix. The apparatus is capable of accommodating four concurrent experiments with rats, and consists of the following basic components: air supply system, animal chambers, ion chambers, vibrating reed electrometers, analog to digital data converters, digital counters, electronic timer, data presentation programmer and digital data printer. In the experiments with MMH-C^{14}, the animal radiorespirometer was modified to determine separately the C^{14} radioactivity in the respiratory $C^{14}O_2$ and in other volatile C^{14} labeled compound(s). Respiratory gases were first passed through a 2N HCl trap, and a drying column to remove any basic compounds. The total C^{14} radioactivity in the respired gases was assayed in a flow ionization chamber. The gas mixture was then passed through a 2N NaOH trap to remove CO_2, a drying column and a second flow ion chamber to determine the radioactivity in any other C^{14} labeled volatile compounds.

SECTION IV

RESULTS AND DISCUSSION

Metabolism of UDMH-C^{14}

Previously, we found (ref. 6) that UDMH-C^{14}, upon administration to rats, can be converted in part to respiratory $C^{14}O_2$ in addition to C^{14} excretion by the kidneys. This study has now been extended to determine the capability of rats to convert UDMH, at different dose levels, to CO_2 over longer periods of time. The time courses for the production of respiratory $C^{14}O_2$ from rats metabolizing UDMH-C^{14} are given in figure 1. Four dose levels have been used in this series of experiments employing rats weighing 250 grams. These levels are: 0.12 mmole/kg, 0.33 mmole/kg, 1.0 mmole/kg and 1.33 mmole/kg. Since the dose level 1.33 mmoles/kg is very close to the previously determined convulsive dose, the reproducibility of the experiment with regard to $C^{14}O_2$ yield was found to be rather poor.

A glance at these time courses enables one to conclude that with lower dose levels the metabolism of UDMH proceeds at a rapid pace, and the decrease of the $C^{14}O_2$ production rate presumably reflects the fact that all the administered UDMH has been exhausted from the site of metabolism, i.e., either by degradation to intermediates and CO_2 or by renal excretion. On the other hand, at the dose level of 1.33 mmoles/kg, the continuing steady $C^{14}O_2$ production indicates that the capability of rats to retain or incorporate UDMH-C^{14} or a metabolite was seriously impaired.

The absolute rate of UDMH metabolism in rats administered with different dose levels of UDMH-C^{14} can be better illustrated in the graph given in figure 2. It can be seen that at dose levels of 1.00 and 1.33 mmoles/kg body weight, the metabolism of UDMH attains a maximum rate, particularly during the first hour after UDMH administration. This maximum

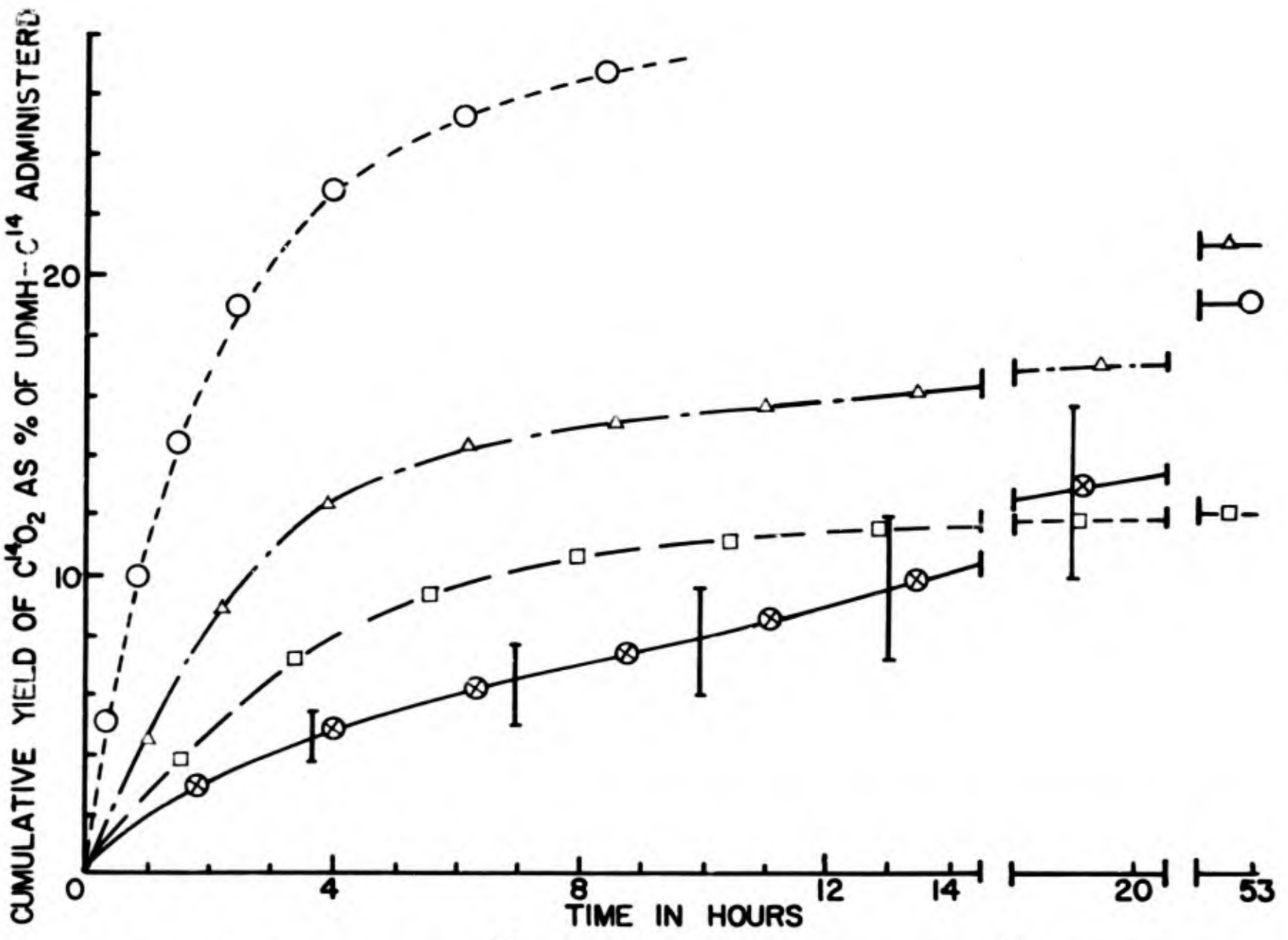

Figure 1. Cumulative yield of $C^{14}O_2$ expressed as percent of UDMH-C^{14} administered. The symbols are as follows: O, 0.013 mmole UDMH/kg, Δ 0.33 mmole UDMH/kg, □ 1.0 mmole UDMH/kg, ⊗ 1.33 mmoles/kg.

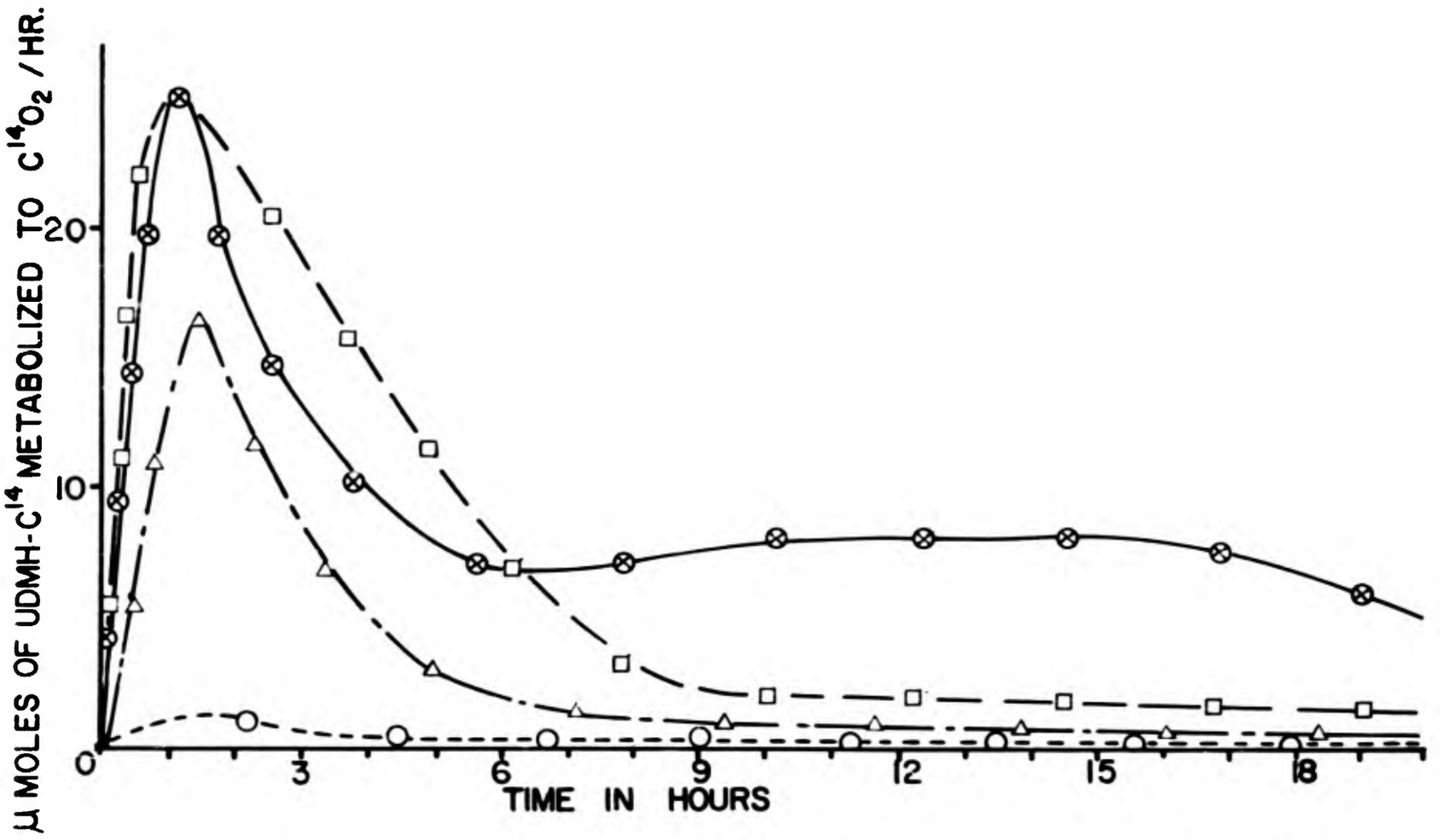

Figure 2. Yield of $C^{14}O_2$ expressed as μmoles of UDMH converted to respiratory $C^{14}O_2$ per hour. The symbols are as follows: ○ 0.013 mmole UDMH/kg, △ 0.33 mmole UDMH/kg □ 1.0 mmole UDMH/kg, ⊗ 1.33 mmoles UDMH/kg.

TABLE I

DISTRIBUTION OF C^{14} IN RESPIRATORY CO_2, URINE, AND TISSUE AFTER ADMINISTRATION OF UDMH-C^{14} TO RATS*

Source	UDMH Dose Level					
	20mg(0.333 mmoles)/kg		60mg(1.00 mmoles)/kg		80mg(1.33mmoles)/kg	
	C^{14} yield percent	mg/kg UDMH equivalent	C^{14} yield percent	mg/kg UDMH equivalent	C^{14} yield percent	mg/kg UDMH equivalent
Urine	56	11.2	53	31.8	70	56.0
$C^{14}O_2$	21	4.2	12	7.2	19	15.2
Retained in tissue**	22	4.4	35	21.0	11	8.8

* Recovery after 53 hours

** This value found by difference

rate presumably reflects the concentration of the enzyme system responsible for the conversion of UDMH methyl carbons to CO_2.

From the findings obtained in the present series of experiments, one can also conclude that the amount of UDMH which can be excreted in urine is dependent upon the dose level of UDMH administered. The conclusion is drawn from the data given in table I. When the findings are expressed as percentage of the administered radioactivity in UDMH-C^{14}, one notes that at the low dose levels from 53-56% of the C^{14} radioactivity was detected in urine. However, when dose level was high, i.e., 1.33 mmoles/kg body weight, the radioactivity in administered UDMH-C^{14} was excreted in urine to the extent of 70%. This may reflect the highly intoxicated state of the animal. While a significant amount of radioactivity in the administered UDMH-C^{14} was apparently retained in the body at 53 hours after administration of 1.33 mmoles/kg, the amount retained was found to be drastically reduced in comparison with lower doses. Here again, it is reasonable to believe that when the dose level employed approaches the convulsive dose there appear to be drastic changes in the metabolism of UDMH by rats. At such a high dose level, the administered UDMH may possibly cause an inhibitory effect upon the mechanism for metabolism of UDMH itself in the rats. The speculation is supported, at least in part, by the fact that some hydrazines have been shown (ref. 7 & 10) to be potent inhibitors of certain amine oxidases.

Back et al (ref. 8) gave evidence for a metabolic change in UDMH after its administration to animals. This was concluded after measuring UDMH levels in blood by the TPF (Trisodium pentacyano amino ferrate) method and by C^{14} tracer techniques. It was found that UDMH levels in blood by the TPF method were only half those determined by the use of UDMH-C^{14}. Since only the C^{14} content of blood was measured, this method did not distinguish between UDMH-C^{14} or a C^{14} labeled metabolite while the TPF method was specific for UDMH. These workers concluded that UDMH was being metabolized and a metabolite was present in blood of treated animals.

The results obtained in the present work, particularly that concerning the production of $C^{14}O_2$ from UDMH-C^{14}, are in line with the contention described by Back et al.

Metabolism of MMH-C^{14}

The time course for the utilization of MMH-C^{14} by intact rats at three different dose levels is given in figure 3. The data are presented as cumulative recovery plots and are expressed as millimoles of volatile compounds. The latter includes respiratory CO_2 on one hand, and a volatile non-CO_2 compound(s) originating from MMH on the other hand. In terms of radiochemical yield, it is evident that a considerably greater amount of MMH has been converted to the non-$C^{14}O_2$ volatile compound in comparison to the production of respiratory $C^{14}O_2$. The non-CO_2 volatile C^{14} labeled compound derived from MMH-C^{14} was found to be a neutral compound and

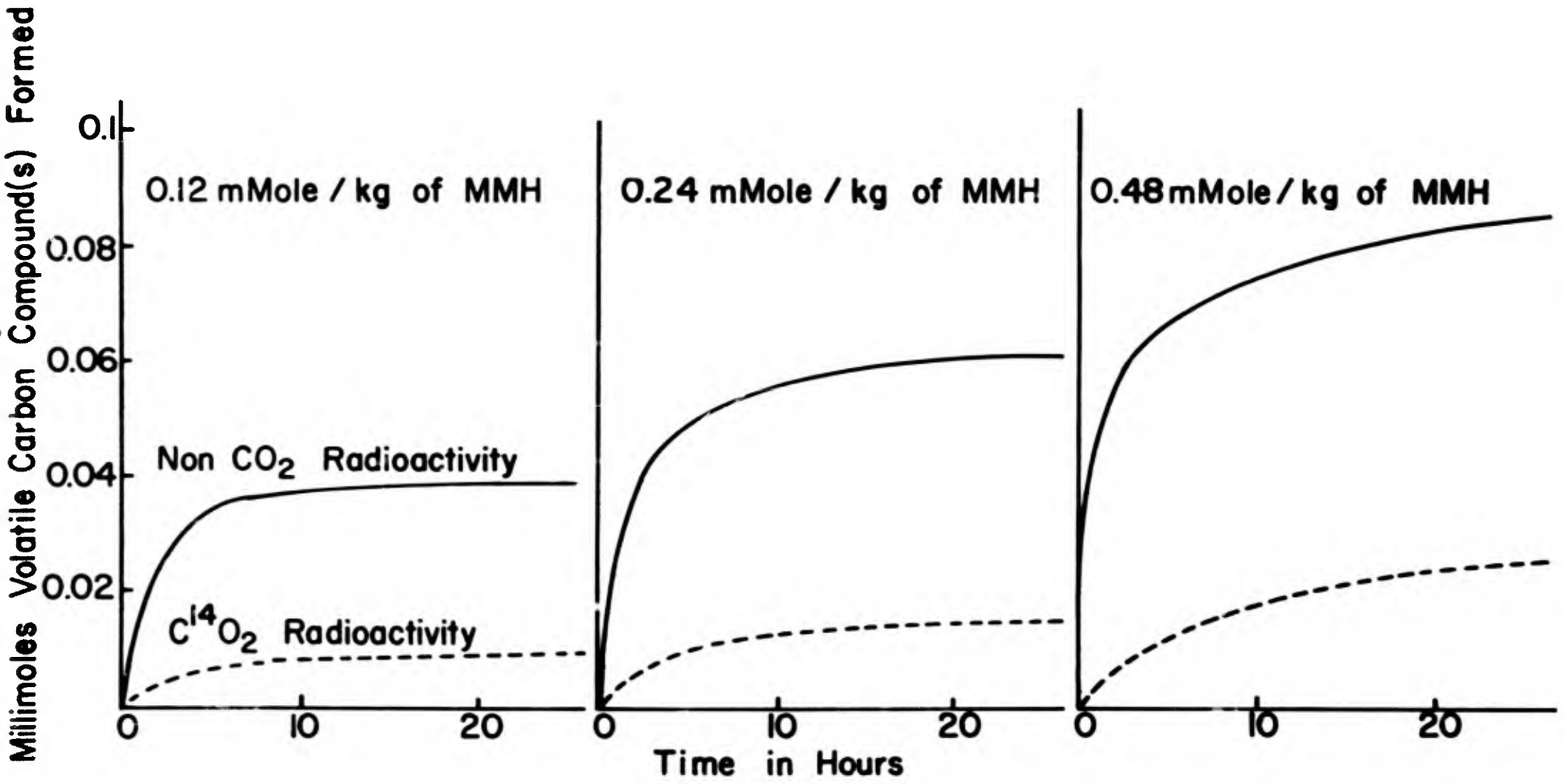

Figure 1. Production of $C^{14}O_2$ and other volatile C^{14} carbon compound(s) by the rat after administration of varying doses of MMH-C^{14}. Data expressed as cumulative millimoles of volatile carbon.

can be condensed at liquid nitrogen temperature. This gaseous compound has been tentatively identified as methane, but the mechanism involved in the conversion of MMH to methane is not yet ascertained. Eberson and Persson (ref. 10) have studied cupric ion catalyzed oxidation of beta phenyl isopropyl hydrazine under conditions resembling those in biological systems. Their data provide evidence for a free radical mechanism leading to the formation of oxidation products which include isopropyl benzene, propenyl benzene, phenyl acetone, 1-phenyl-2-propanol and beta phenyl-2-propanol. Beaven and White (ref. 11) have demonstrated that interaction of phenylhydrazine and oxyhemoglobin produced benzene and molecular nitrogen in the presence of oxygen. Newman and Nadeau (ref. 12) have reported the formation of methane, nitrogen and small quantities of carbon monoxide from a dilute aqueous solution of MMH upon oxidation by sodium hypochlorite. From these studies, it is evident that monoalkylhydrazines are readily oxidized by many relatively weak oxidizing agents. The products of oxidation appear to be a result of free radical mechanism and, in the case of MMH, would result in the formation of methane. The kinetics for the production of $C^{14}O_2$ and the non-CO_2 volatile compound can be also examined from the plots given in figures 4 and 5. In this case, the data are plotted as hourly recovery of the respective volatile compounds and expressed as micromoles of MMH converted. In the case of non-CO_2 volatile compounds from MMH, the active production occurred primarily during the first 2 hours regardless of dose level of MMH. On the other hand, the production of $C^{14}O_2$ from MMH-C^{14} reached a peak rate during the first two hours and then remained elevated for a longer period of time, particularly in the case of higher dose levels. The observed contrast suggests that different mechanisms are involved in the production of respiratory CO_2 and non-CO_2 volatile compound from the administered MMH.

A C^{14} distribution inventory of administered MMH-C^{14} in rats is given in table II. The amounts of C^{14} retained in tissue were obtained by difference. It is noted that at lower dose levels the renal excretion of MMH or its derivative(s) was proportional to the administered doses. However, when dose levels reached 0.48 mmole/kg body weight, the absolute amount of MMH or its derivative(s) excreted in urine was essentially the same as that observed at 0.24 mmole/kg body weight. This fact implies that there exists a limit in the excretion rate of MMH or its derivative(s). In accordance with this latter observation, one also finds at a 0.48 mmole/kg dose, that a considerably greater amount of MMH-C^{14} radioactivity was retained in the tissue at 27 hours when the experiment ended. At a high dose level, the excretion capabilities of the rat possibly may have been jeopardized by the serious state of intoxication. On the other hand, the absolute amount of $C^{14}O_2$ produced and the amount of non-CO_2 volatile compound produced appears to be proportional, within limits, to the dose level administered. This may indicate that the metabolic capability is not affected seriously by higher dose levels of MMH despite reported inhibition of monoamine oxidases by hydrazines (ref. 1 & 7).

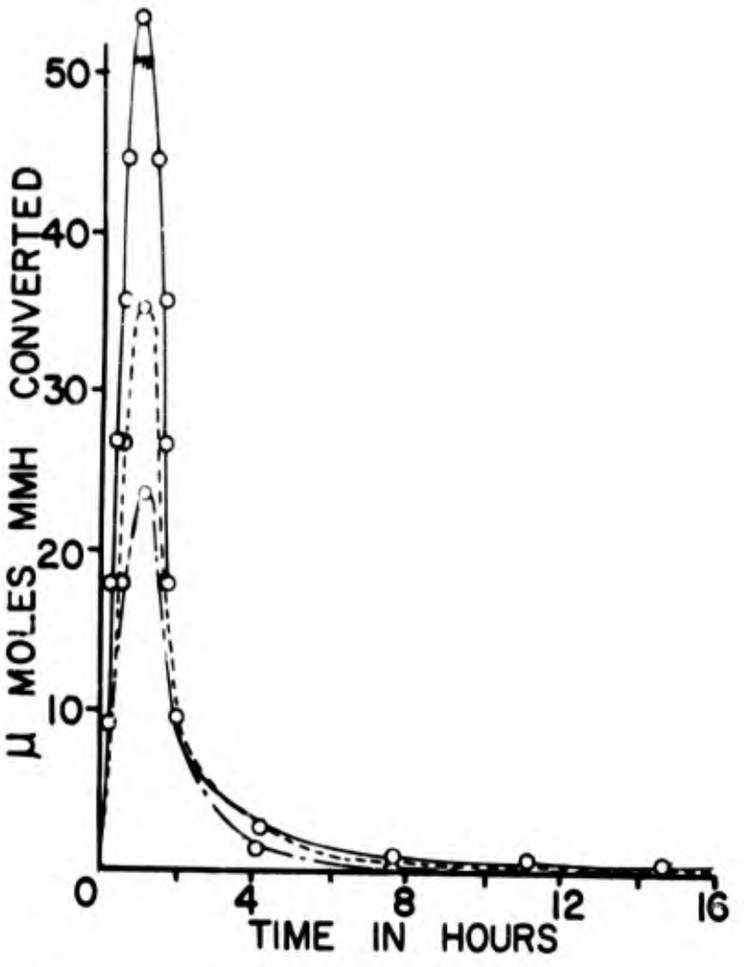

Figure 4 Formation of non-$C^{14}O_2$ volatile C^{14} labeled compound(s) from different dose levels of MMH-C^{14}. The curves are designated as follows: ____ 0.48 mmole MMH/kg, - - - 0.24 mmole MMH/kg, __ . __ 0.12 mmole MMH/kg.

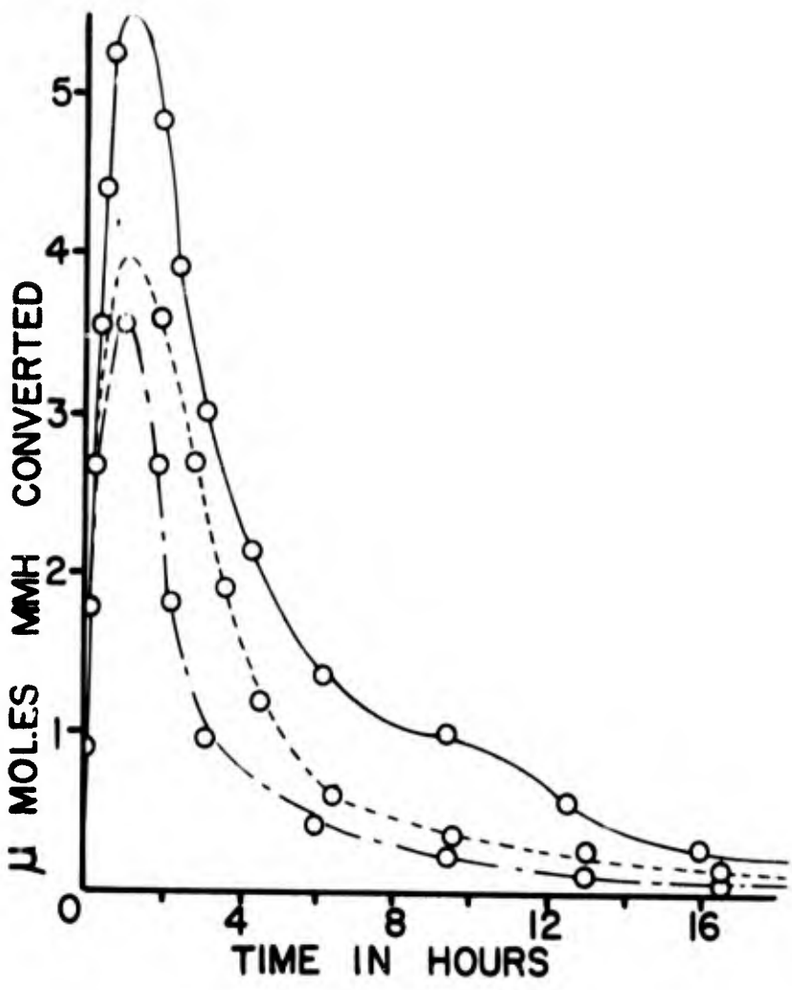

Figure 5. Formation of $C^{14}O_2$ from different dose levels of MMH-C^{14}. The curves are designated as follows: ____ 0.48 mmole MMH/kg, - - - 0.24 mmole MMH/kg, __ · __ 0.12 mmole MMH/kg.

TABLE II

DISTRIBUTION OF C^{14} IN RESPIRATORY GASES,

URINE, AND TISSUES AFTER ADMINISTRATION OF MMH-C^{14} TO RATS

Recovery after 27 hours *

Source	MMH Dose Level					
	5.5 mg(0.12mmole)/kg		11 mg (0.24mmole)/kg		22 mg (0.48mmole)/kg	
	C^{14} yield percent	mg/kg MMH equivalent	C^{14} yield percent	mg/kg MMH equivalent	C^{14} yield percent	mg/kg MMH equivalent
Urine	41	2.26	39	4.29	22	4.84
$C^{14}O_2$	8	0.44	7	0.77	5	1.10
Non - $C^{14}O_2$ respiratory C^{14}	26	1.43	20	2.20	18	3.96
Retained in tissue+	25	1.37	34	3.74	55	12.10

* Values are the average of two rats

\+ Determined by difference

APPENDIX

DESIGN AND PERFORMANCE DATA OF AN ANIMAL RADIORESPIROMETER

Basic Design Concept

The term "radiorespirometry" refers to a type of experiment designed to collect kinetic information on the rate and extent of production of respiratory $C^{14}O_2$ from an animal metabolizing a C^{14} labeled substrate. The apparatus required for this type of experiment, i.e., an animal radiorespirometer, must be capable of fulfilling the following requirements:

1. A controlled air flow system so that a defined amount of air can be passed through the animal chamber and the detection apparatus.

2. A convenient method of radioactivity detection so that one can continuously monitor the amount of $C^{14}O_2$ in the air of an animal chamber.

3. A good detection sensitivity in measuring $C^{14}O_2$ in a stream of flowing air. In order to follow rapid metabolic events, the flow rate involved may be as high as a few liters a minute.

4. Minimum drift of detection efficiency so that the detecting device does not have to be calibrated at frequent intervals.

With these requirements in mind, one finds that the most suitable detecting device for an animal radiorespirometer is a flow ion chamber equipped with a vibrating reed electrometer for measuring the current output of the ion chamber. Moreover, to facilitate data processing, it is advisable to translate the analog information from the electrometer to digital form. The animal radiorespirometer currently in use in this laboratory was designed and constructed in accordance with the foregoing considerations. Four parallel systems were constructed to accommodate four concurrent radiorespirometric experiments.

Animal Radiorespirometer Components

The air flow scheme and the block diagram of various components of the radiorespirometer are shown in figures 6 and 7, respectively. The components for the entire apparatus are briefly described in the following:

The Gast pumps, Model V-70572, employed in the air flow system were manufactured by the Brentwood Company, Brentwood, Missouri. The animal chambers, air reservoir, manifolds and NaOH traps were fabricated at Oregon State University. The flow meters, Model 90144 B, were manufactured by the Emil Greiner Company, New York City, New York. The

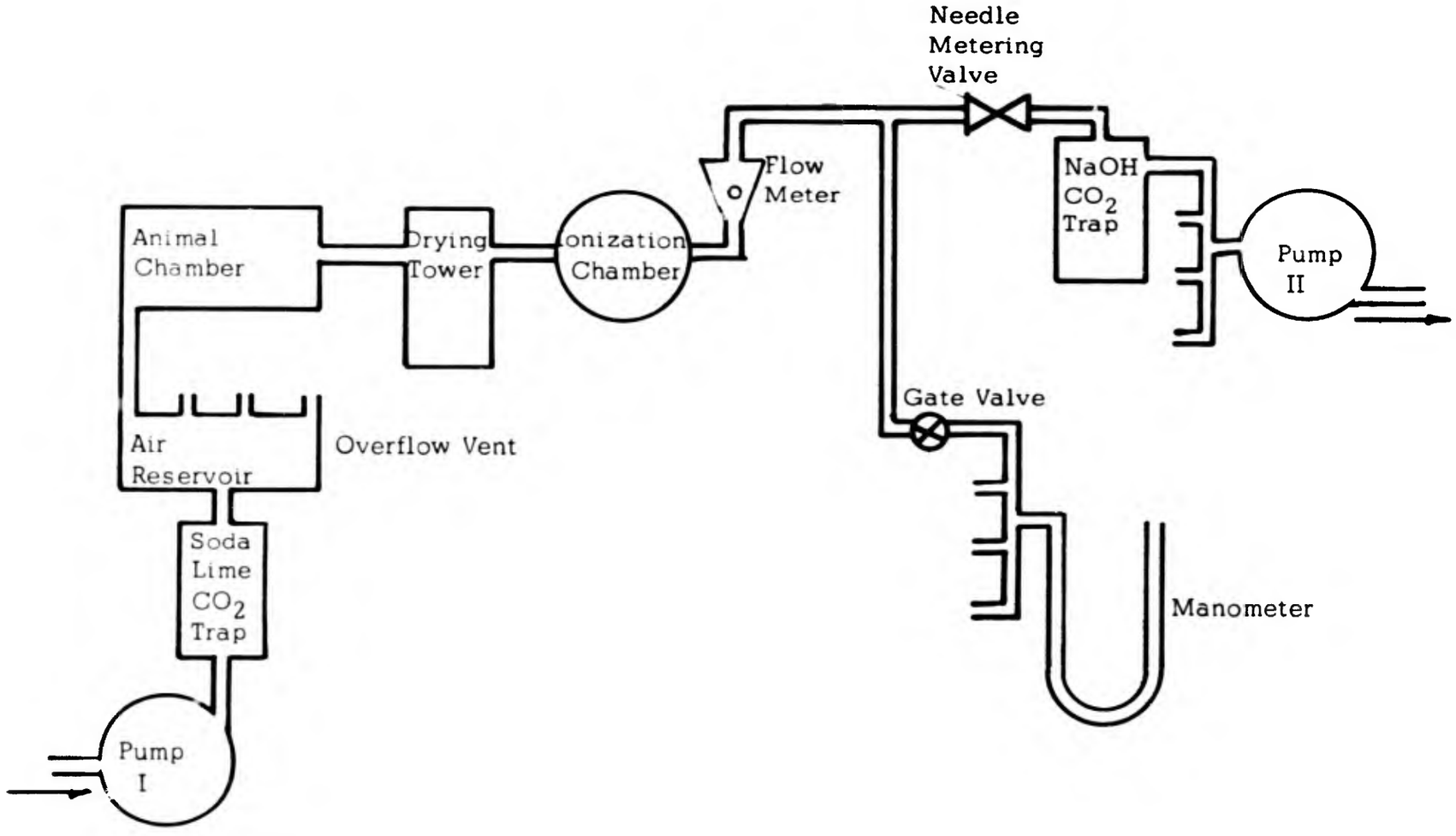

Figure 6. Air flow diagram for animal radiorespirometry system.

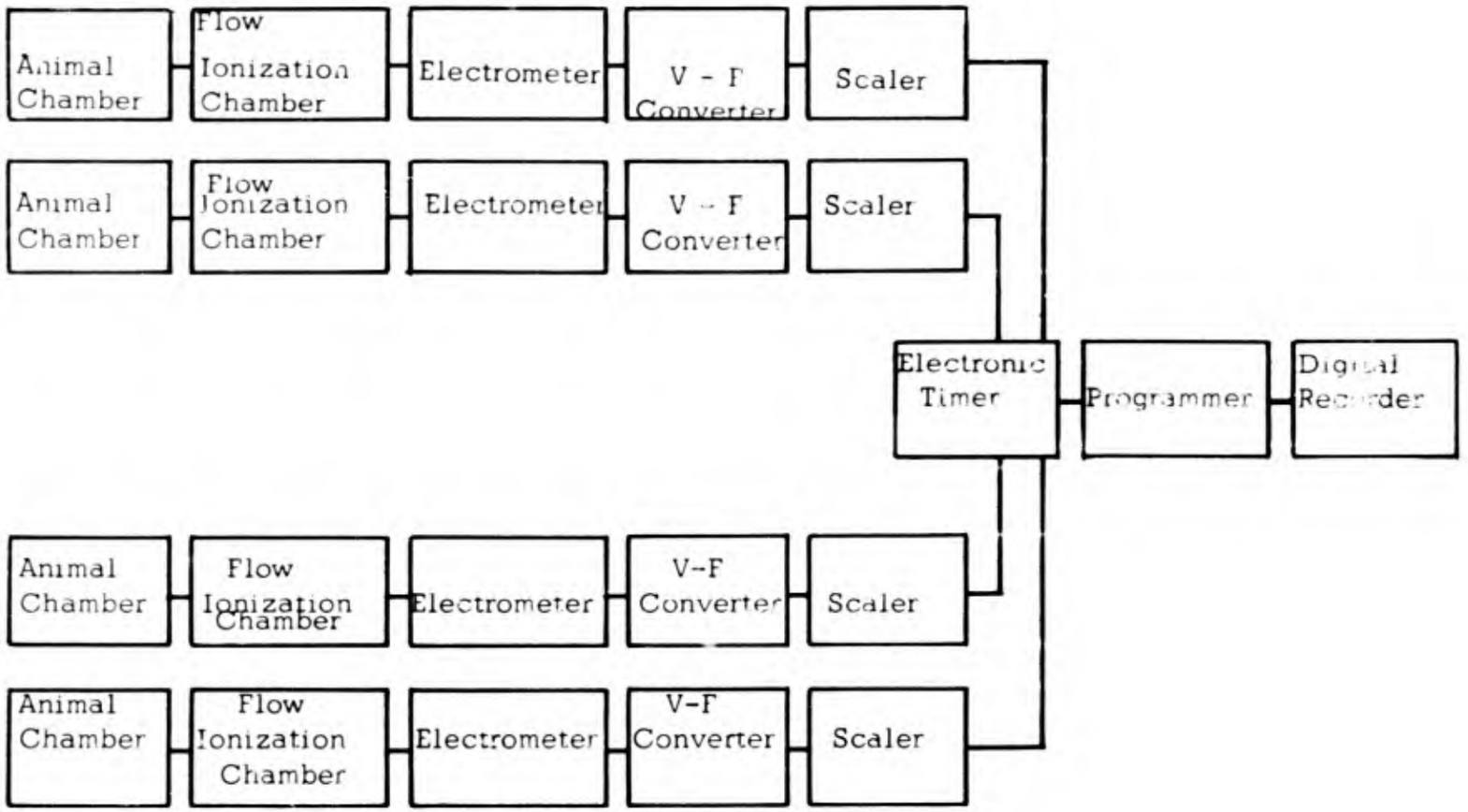

Figure 7. Block diagram of animal respirometer with printout system.

needle valves are Nupro Fine Metering Valves, Model B-4MA, manufactured by Nuclear Products Company, Cleveland, Ohio. The gate valves are Whitey valves, Model 1-V S4-B, manufactured by Whitey Research Tool Company, Oakland, California. The one-liter flow ionization chambers and Model 31 Cary electrometers were supplied by the Applied Physics Corporation, Monrovia, California. Each electrometer was modified to provide a 0-30 volt output. The Dymec V-F Converters, Model 2210-R and the digital printer Model 562-A were manufactured by Hewlett-Packard Corporation, Palo Alto, California. The RIDL six-decade scalers, Model 49-43; instrument case and power supplies, Model 29-1; the electronic timer, Model 54-8; and programmer, Model 52-44; were manufactured by the Radiation Instrument and Development Laboratory, Melrose Park, Illinois. The input requirements of the RIDI scalers were modified to match the output of the Dymec V-F Converters.

All polyethylene tubing fittings were Swage-Lok fittings manufactured by the Crawford Fitting Company, Cleveland, Ohio.

The Air Flow Scheme

As shown in figure 6, air under positive pressure is passed through soda lime columns to remove atmospheric CO_2, and into a vented reservoir maintained at a slightly positive pressure. A pump located at the downstream end of the circulation system provides slight negative pressure to pull the CO_2-free air from the reservoir through the four animal chambers.

From each of the four animal chambers, air and respiratory gases pass through a drying tower, then through a 1 liter stainless steel ionization chamber. The air stream is then passed through 2N NaOH to remove $C^{14}O_2$ and vented to the atmosphere. Pressure in the animal chambers and ionization chambers is maintained at approximately 5 mm and 10 mm H_2O negative to atmosphere, respectively. A water filled manometer is employed to monitor the pressure throughout the entire system.

The Radioactivity Measurement System

As shown in figure 7, radioactivity in the flowing gas mixture is detected by the ionization chamber of each channel operating at a collection voltage of 45 volts. The current produced by collection of electrons in the chamber is amplified by an electrometer whose output is put into a voltage-to-frequency converter. The digital output of the converter is accumulated and displayed by the six decade scaler. Since the voltage output of the electrometer is directly proportional to the amount of radioactivity in the ionization chamber at any given instant, the number of digits displayed (10,000 per second at full scale) per unit time relates directly to the amount of radioactivity measured during that time period. Selective interval timing is provided by the electronic timer. The digital information displayed on the four scalers and the timer is recorded by the digital recorder. The timing,

scaler interogation and recording are integrated by the electronic programmer.

Performance Data

In order to evaluate the capabilities of the animal radiorespirometer, a series of tests have been made to obtain information on the performance of the entire system, including the radioactive detection system.

"Response Time" for the Detection of $C^{14}O_2$ Originating from the Animal Chamber

Inasmuch as the dead volume of the entire radiorespirometer system, particularly between the animal chamber and the ionization chamber, is of the magnitude of liters, it requires time for sweeping air to carry the $C^{14}O_2$ originated in the animal chamber into the ion chamber where radioactivity measurement can be made. The time involved, designated as "response time" is naturally dependent not only on the volume of the system but also on the flow rate of the sweeping air. In the present case, the volume of the animal chamber, drying tube, and the ion chamber are kept constant. Consequently, one need only to measure the response time as a factor of the air flow rate. The response time in reality involves two elements; one is the initial response and the other is the time required to reach saturation level of radioactivity, in a defined amount of $C^{14}O_2$ containing air, passing through the ion chamber. In the latter case, the "saturation response time" has been determined at various rates of air flow and the results are given in figure 8. It can be seen that when the air flow rate is 500 ml/min., 10 minutes are required before saturation response can be realized. However, it should be stressed that the initial response time at this flow rate is of the order of 30 seconds.

Radioactivity Detection Efficiency

The radioactivity detection efficiency is dependent upon three basic parameters. These are the size of the ion chamber, the flow rate of sweeping air and the background radioactivity level.

It is generally understood that when the size of the ion chamber is smaller than 100 ml, there is a drastic reduction of detection efficiency. For this reason, in the animal radiorespirometer, 1,000 ml ion chambers were employed. With ion chambers of this size, the detection efficiency for a static sample of $C^{14}O_2$ in the chamber is approaching 100%.

The background of the detection system is derived to a very limited extent from the electronic noise in the electrometer circuit, the greater portion results from external radiation originating from either cosmic radiation or alpha emitters such as radon in the sweeping air. With ion chambers 1,000 ml in size, it has been determined that approximately 120-140

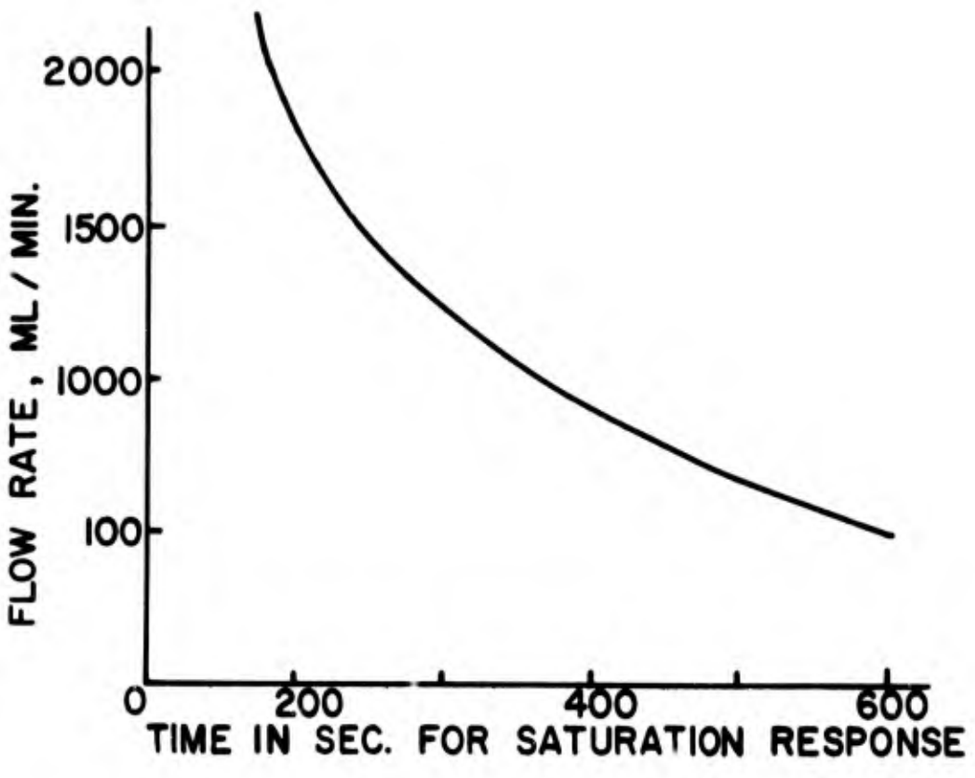

Figure 8. Relation of radiorespirometer response time to flow rate, using 1 liter ionization chamber.

alpha particles can be detected per hour. This alpha radioactivity, in addition to the other background events, produces a total background current from the radioactivity detection system equivalent to approximately 2,000 digits per minute in the digital read-out when the electrometers are used with a 300 millivolt full scale range setting. Meanwhile, it has also been found that with 1,000 ml ion chambers and at an air flow rate of 500 ml per minute, 1 μc of $C^{14}O_2$ will give rise to 2×10^{-7} digits. This fact implies that if 1 mμc of $C^{14}O_2$ is produced by an animal per minute, the current produced in the ion chamber will be 10 times greater than the background radioactivity level. Taking into consideration the stability of the electrometer system, such a level of radioactivity can be determined with good reliability. One can therefore conclude that the animal radiorespirometer so designed can accommodate experiments with animals administered as little as 1μc of C^{14} labeled substrate, provided the substrate can be utilized by the animal to some extent.

Stability of the Radioactivity Detection System

The stability of the entire system is determined by two parameters. First, the voltage-to-frequency converter has been found to display excellent stability and does not drift more than .03% of full scale, over a period of one day. Second, the electrometer system has been determined to have a drift characteristic of less than 4 μv/second, a value which produces no measurable drift in a 24 hour radiorespirometer experiment.

Taking this parameter into consideration, it can be readily seen that the reproducibility of the radioactivity measurement hinges on the ratio of radioactivity level to background level. As previously indicated, the average background level is approximately 2,000 digits/min. The average deviation of background from the mean value was found to be 380 digits/min. On the basis of this result, it can be readily concluded that when the radioactivity level to be measured is 5 times background level, one would expect a reproducibility of a radioactive measurement on the order of 5%. It should be stressed that when the experiment is properly designed the level of radioactivity in the respiratory $C^{14}O_2$ during the active phase of the substrate metabolism is generally greater than 100 times the background level. This implies that the reproducibility for radioactive measurement does not constitute a use limiting factor with regard to the reproducibility of the radiorespirometric experiment.

Calibration of Radioactivity Detection System

In order to provide absolute calibration, the digital equivalent to a carefully measured amount of radioactivity as $C^{14}O_2$ was established in the following manner: The volume of each ionization chamber between its stopcocks was determined by weighing when empty and filled at 25° C with distilled water. The volume of all chambers was within 0.2% of 1022 ml in volume. After

drying the chambers, a gas containing $C^{14}O_2$ in an approximately known concentration was passed through each chamber until the digital response to radioactivity reached a maximum.

Digital response was then observed and recorded over periods of static and flowing operation. Each chamber was then flushed with dried air through 30 ml of ethanolamine ethanol (2:1 v/v) at 30 ml/min to trap all C^{14} contained in the known volume of the ionization chamber. After flushing, the trapping solution was brought to volume and aliquots were assayed for C^{14} by liquid scintillation counting techniques.

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13 ABSTRACT

Many of the applications of hydrazines, especially as rocket propellants and in medicine, give them considerable toxicological importance. The respiratory and urinary excretion by rats of unsymmetrical dimethylhydrazine (UDMH) and monomethylhydrazine (MMH) and their metabolites has been studied by means of radiotracer techniques. At a very low dose, almost 30% of the C^{14} appeared as respiratory $C^{14}O_2$ in 10 hours. At a convulsive dose, the conversion of UDMH-C^{14} to $C^{14}O_2$ amounted to slightly greater than 13% at the end of 20 hours. At all doses studied radioactivity appeared in the urine to the extent of at least 50% of the administered UDMH-C^{14}, at the end of two days after administration. Rats administered MMH-C^{14} by i.p. injection at 20% of a median lethal dose respired approximately 45% of the administered radioactivity in 24 hours. The respired radioactivity consisted of at least two components; 20-25% was $C^{14}O_2$, and the remainder was a C^{14} labeled volatile compound tentatively identified as methane-C^{14}. At the sub-convulsive doses, 40% of the administered radioactivity in MMH-C^{14} was excreted in urine. At a toxic dose the percentage of urinary excretion of C^{14} decreased, but net molar excretion increased slightly. The design of an animal radio-respirometric system capable of continuously monitoring C^{14} in respiratory gases from separate animals is described.

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Respiratory $C^{14}O_2$						
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