GAS CHROMATOGRAPHIC ANALYSIS OF BODY FLUIDS FOLLOWING MIDLETHAL IRRADIATION

Final Report

Arthur L. Gross
George K. Kibler

Project 05-1630
Contract No. AF 41(609)-2676
Task No. 775702

to

USAF School of Aerospace Medicine
Aerospace Medical Division (AFSC)
Attn: SMSPP
Brooks Air Force Base, Texas 78235

15 December 1965

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APPROVED:

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I. INTRODUCTION

This is the final report on Contract No. AF 41(609)-2676, a program dealing with the gas chromatographic analysis of body fluids of normal and irradiated laboratory animals. The program was organized to utilize existing gas chromatographic procedures in current use at the laboratories of Southwest Research Institute for screening a large number of chemical substances to determine what alterations result from radiation exposure.

II. WORK PERFORMED

During the course of the program rats were irradiated, blood or urine was collected from the animals, and gas chromatographic analyses were performed using three different methods that were currently in use in the Institute's laboratories. During the last quarterly period of the program monkeys were used as the experimental animals. They were irradiated and blood samples periodically taken from the animals were analyzed for amino acids.

A. Irradiation of Animals

A Cobalt 60 source located at Southwest Research Institute was used for irradiating the animals. The cobalt is physically contained in tapes whose geometrical configuration is calculated in order to regulate dosimetry. The initial work that was conducted with the cobalt was based on a dose rate of 100R per minute. The animals during the first quarterly period of the program received 500R of total body radiation. This was confirmed using an alanine dosimeter that was developed at Southwest Research Institute. As the program progressed the animals received radiation dosages as high as 5000R. During the last quarterly period of the program Macaca irus monkeys were used. These animals received 2500R of total body radiation that was administered during a period of forty minutes.

Locally obtained albino rats (225-250 grams) were used during the

initial phases of the program during which time irradiation parameters were established and blood sampling techniques were selected. The irradiation experiments were conducted on Sprague-Dawley rats obtained from Sprague-Dawley Incorporated, Madison, Wisconsin. The gas chromatographic analyses were performed on blood obtained by heart puncture at set intervals after irradiation. Initially blood was drawn immediately after irradiation but this was later changed to also include samples drawn up to twenty-four hours after irradiation. During the third quarterly period the analyses were performed on urine samples that were obtained directly from the urinary bladder by means of a hypodermic syringe. A total of 115 rats were used to conduct the work.

Blood samples were taken from the monkeys prior to irradiation and served as controls. After irradiation blood was taken from the monkeys every second day until the animals reached a fairly advanced stage of radiation sickness when they were sacrificed. A total of three monkeys were used during this phase of the program.

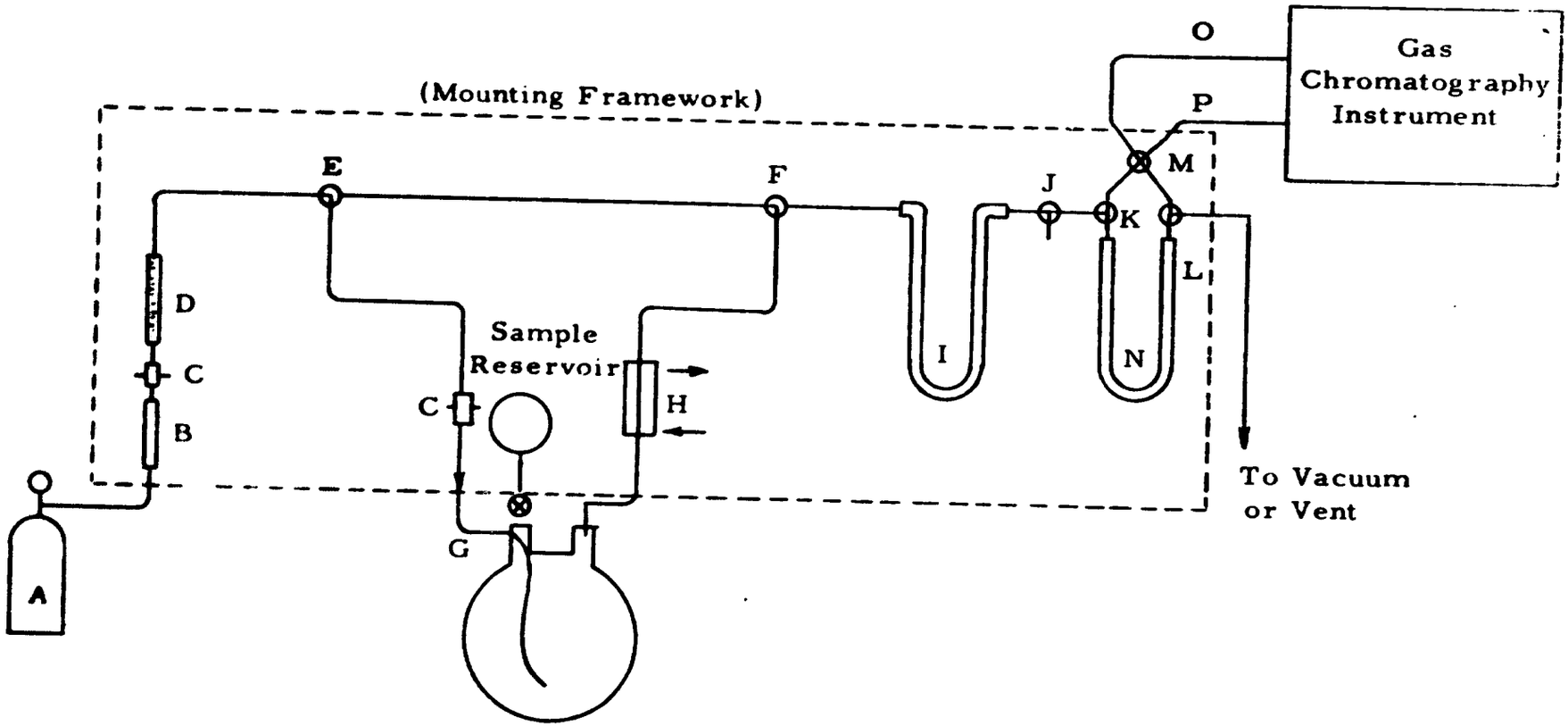
B. Analytical Methods

Three analytical methods were used throughout most of the program. These include a vacuum stripping procedure that measures lower molecular weight volatile substances, a steam distillation stripping that measures higher boiling substances, and an extraction procedure coupled with both microcoulometric detection and hydrogen flame ionization detection. Amino acid analyses were performed using a gas chromatographic

procedure developed at Southwest Research Institute by Dr. Donald E. Johnson and co-workers. All of these methods are described below.

C. Vacuum Stripping Method

The vacuum stripping method was originally developed for the analysis of coffee volatiles, and was adapted for use in the analysis of body fluids on a previous contract with the School of Aerospace Medicine (Contract No. AF4-(657)-281). It was further modified to its present form which is shown schematically in Figure 1. The method consists of a stripping step in which the sample passes through a small aperture into a chamber at reduced pressure. The sample is essentially atomized allowing the volatile substances to more readily boil or flash out of the minute droplets. The flash chamber is at a reduced pressure of 40 mm mercury and heat is applied so that the aqueous sample boils at 37°C. During the flashing step helium flows through the system at a flow rate of approximately 500 ml per minute. This is fixed by a capillary restriction which allows approximately 6 ml per minute of helium to flow against atmospheric pressure and at a tank pressure of 12 psi. The helium serves to sweep the volatiles and water out of the flashing chamber. The volatiles and a small amount of water are trapped on a preliminary gas chromatographic column immersed in liquid nitrogen. The preliminary column is then heated at 60°C and the volatiles are transferred by means of a chromatographic separation to a freeze-out trap immersed in liquid nitrogen. Water that has been trapped in the preliminary gas



A - Helium supply
B - Drying tube
C - Capillary to limit flow
D - Flowmeter
G - Helium inlet to flashing chamber
H - Ice water condenser

E, F, J, K, L - 3-way stopcocks
M - 4-way stopcock
I - Preliminary column (7 mm tubing X 10 inches packed with Carbowax 1500-C-22 firebrick)
N - Freezeout trap (packed with glass beads)
O - Helium carrier gas line from reference side of detector
P - Connection to chromatographic column

FIGURE 1. VACUUM STRIPPING APPARATUS

chromatographic column is vented to the atmosphere. The freeze-out trap is then heated and the volatiles are picked up by a carrier gas and transferred to the gas chromatographic instrument for analysis. The entire method takes approximately 20 minutes from the time of flashing until the sample is transferred to the gas chromatographic instrument. A photograph of the vacuum stripping apparatus is shown in Figure 2.

The column used for the analysis of the volatile substances was three meters in length and composed of 11.5% Carbowax 1500 and 88.5% alkali washed Chromosorb-P. Helium was the carrier gas and the column was operated at 50°C in a Barber Colman Model 20 gas chromatograph equipped with a hydrogen flame ionization detector.

D. Steam Distillation Method

This method was originally developed to concentrate the higher boiling substances in food products that are flavor constituents. The method is shown schematically in Figure 3. Essentially it is a modification of the vacuum stripping method: the basic difference being that water and higher boiling substances are not returned to the sample flask by an ice water condenser. Instead, the sample is first trapped in the ice water condenser along with water that boils from the sample flask. As in the vacuum stripping method, the system is operated at reduced pressure so that water boils at approximately 37°C. The condensed sample is then transferred to a condensate holder-extractor shown in Figure 4 and subjected to a diethyl ether extraction. The ether extract is injected into

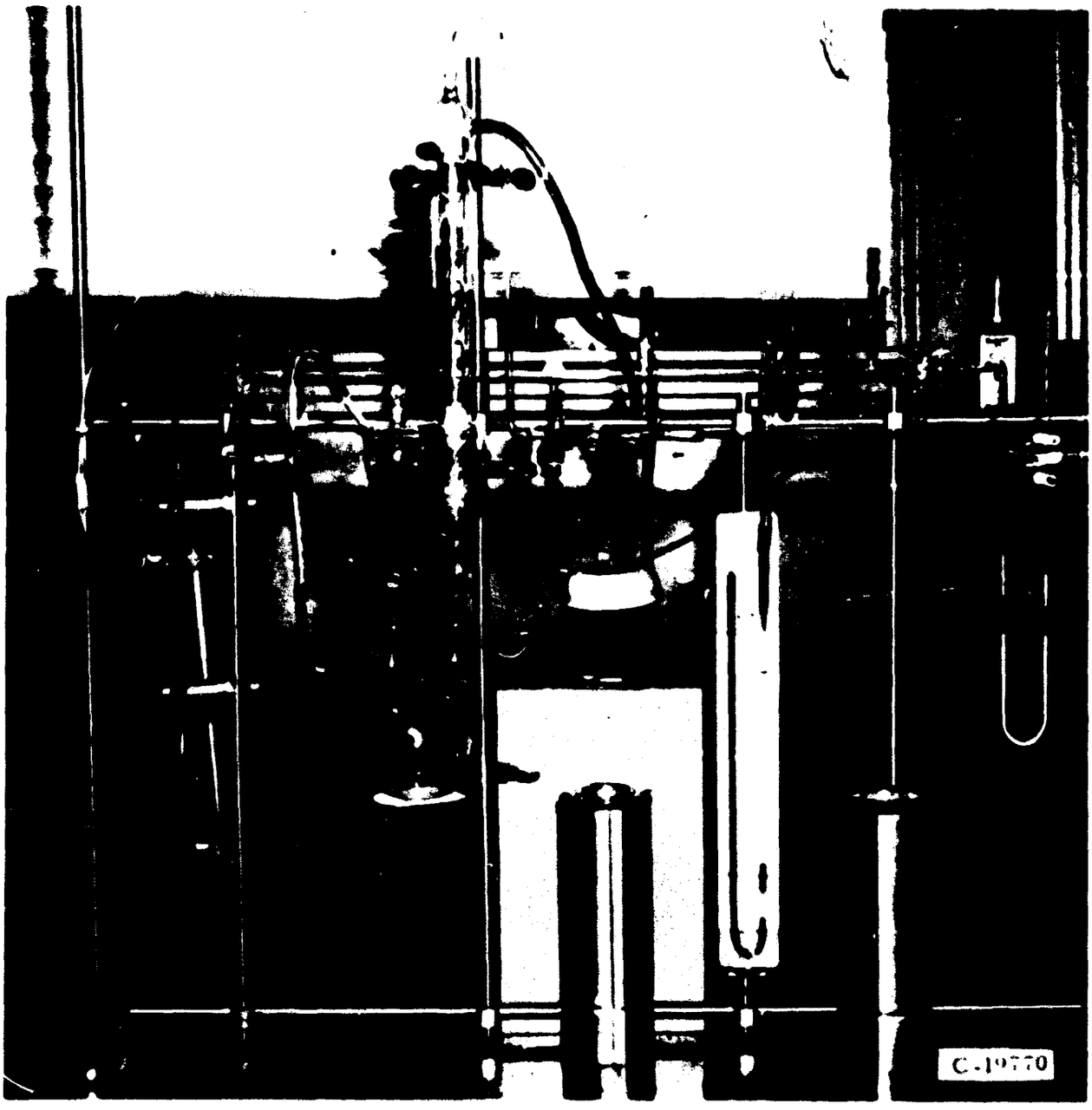
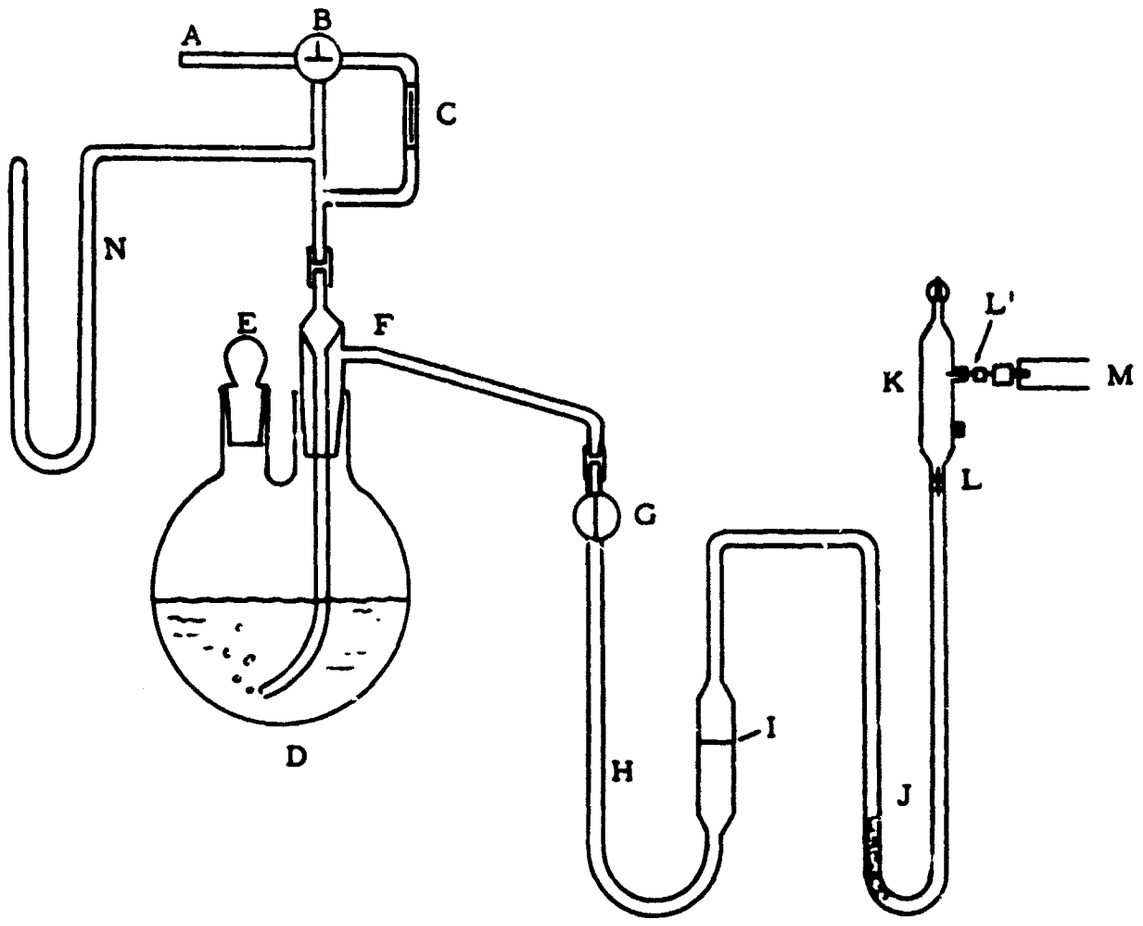


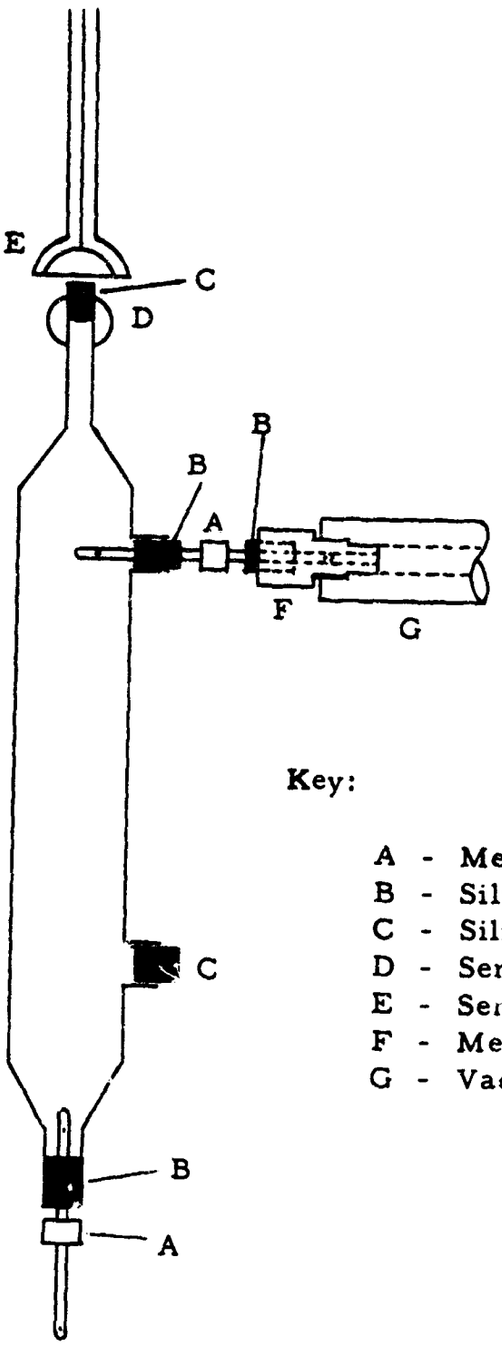
FIGURE 2. PHOTOGRAPH OF VACUUM STRIPPING APPARATUS



Key:

A	-	Entry for sweep gas
B	-	Stopcock, 3-way
C	-	Capillary restriction, approx. 3 ml per min at atm. pr.
D	-	Flask, 300-ml, 2-neck, $\overline{S}$24/40
E	-	Glass stopper
F	-	Head with gas entry and exit for gas and volatiles
G	-	Stopcock
H	-	Condenser, ice water cooled
I	-	Calibration mark
J	-	Condenser, liquid nitrogen cooled, containing glass wool
K	-	Condensate holder-extractor
L, L'	-	Metal connectors, Burrell (greaseless valves)
M	-	Vacuum tubing to bleed control and vacuum pump
N	-	Closed-end Hg manometer

FIGURE 3. STRIPPING ASSEMBLY



Key:

A - Metal connectors, Burrell
B - Silicone rubber plug, 1-hole, Burrell
C - Silicone rubber plug, solid, Burrell
D - Semiball joint, 12/5
E - Semiball joint, 12/2
F - Metal socket, Burrell
G - Vacuum hose

FIGURE 4. CONDENSATE HOLDER-EXTRACTOR

a gas chromatographic instrument. The column used to analyze these substances was 4% SE-30 and 0. 25% Carbowax 20M on Anakrom ABS 80-90 mesh. An F&M Model 400 gas chromatograph was used and was temperature programmed up to 240°C.

E. Microcoulometric Gas Chromatography

The microcoulometric detector is highly specific for the detection of compounds containing halogen or sulfur. During the course of the program dichloromethane (DCM) extracts were made from blood samples, chromatographed and detected with a sulfur detecting microcoulometer. The DCM extracts were also chromatographed on the other instruments used in this program.

F. Amico Acid Analysis

A protein free filtrate is prepared from the blood sample using the conventional tungstic acid method. Sulphate ion is removed with barium hydroxide. The amino acids are then esterified with n-amyl alcohol and acylated with trifluoroacetic anhydride. The sample is then concentrated in a rotary flash evaporator and is ultimately dissolved in dichloromethane. It is chromatographed on a one meter column of 4% SE-30 on Anakrom ABS at temperature that is programmed from 80°C to 230°C.

III. RESULTS

The results of all the analyses failed to demonstrate any changes in body fluid chemistry that could be in any way related to irradiation of the experimental animals. The compounds that were observed include a wide spectrum of aldehydes, ketones, alcohols, thioethers, lactones, more than twenty amino acids, and a great many compounds that were not identified.

IV. CONCLUSIONS AND RECOMMENDATIONS

It must be concluded that the analytical methods that were used, hence the substances that were observed, did not quantitatively or qualitatively change in any way that could be related to the irradiation received by the experimental animals.

It is recommended that future work be conducted on more specific biochemical systems rather than the "broad" approach that was used. A most promising area is that of measuring nucleic acid metabolites in body fluids as a function of radiation damage. A number of papers have been recently published that describe changes in nucleic acid metabolism resulting from radiation damage, which when further investigated may prove to be rewarding. Since gas chromatographic methods now exist that could be used to measure changes in nucleotide and nucleoside composition of body fluids, it is recommended that this program be extended to this area.