DNA 3002T
February 1973

ORGANIC ACIDS AS METABOLIC INDICATORS

THE METABOLISM OF ^{14}C-PROPIONATE IN RATS EXPOSED TO IRRADIATION AND THERMAL INJURIES

HEADQUARTERS
Defense Nuclear Agency
Washington, D.C. 20305

PREPARING AGENCY
Lovelace Foundation
for
Medical Education and Research
5200 Gibson Boulevard SE
Albuquerque, New Mexico 87108
Contract No. DA-49-146-XZ-359

DNA 3002T
February 1973

ORGANIC ACIDS AS METABOLIC INDICATORS

THE METABOLISM OF ^{14}C-PROPIONATE IN RATS EXPOSED TO IRRADIATION AND THERMAL INJURIES

Thomas R. Henderson and Robert K. Jones

THIS WORK WAS SUPPORTED BY THE DEFENSE NUCLEAR AGENCY
UNDER NWER SUBTASK MA112-03

HEADQUARTERS
Defense Nuclear Agency
Washington, D.C. 20305

PREPARING AGENCY
Lovelace Foundation
for
Medical Education and Research
5200 Gibson Boulevard SE
Albuquerque, New Mexico 87108
Contract No. DA-49-146-XZ-359

Destroy this report when it is no longer needed.
Do not return to sender.

THIS PAGE IS INTENTIONALLY LEFT BLANK.

ABSTRACT

^{14}C-propionate utilization was found to be a useful in vivo indicator of the severity of burn or irradiation injuries and the extent of recovery from such injuries. Flash burns (9.2 cal/cm^2, 30 percent of body area) or gamma irradiation (750 rad) produced a significantly reduced rate of $^{14}CO_2$ production from 1-^{14}C-propionate during the initial shock phase following exposure. Recovery from nonlethal injuries was associated with recovery of ability to oxidize propionate within 1 to 2 weeks. In the case of animals exposed to irradiation plus flash burns, a greater initial decrease in propionate oxidation was noted, and recovery did not occur. The persistence of decreased rates of propionate oxidation often was correlated with early mortality.

Analysis of urinary organic acid excretion patterns showed that healthy rats excreted mainly ^{14}C-citrate after being injected with ^{14}C-propionate. Following exposure to injury, the excretion of ^{14}C-citrate was reduced markedly. Recovery of citrate excretion to normal ensued in 2 to 7 days in animals exposed to nonlethal flash burns or gamma irradiation alone, but little recovery was noted in animals exposed to both injuries.

It was concluded that a deficiency of Krebs' cycle intermediates was a significant characteristic of the initial metabolic state or "ebb" phase following injuries and that biochemical events sensitive to irradiation were involved in recovery. Measurements of the rate and pathway of propionate utilization appeared to be a useful physiological monitor for estimating the extent of recovery from thermal burns.

THIS PAGE IS INTENTIONALLY LEFT BLANK.

ACKNOWLEDGMENTS

This project was supported by the Defense Nuclear Agency, Department of Defense, under Contract No. DA-49-146-XZ-359. We wish to express our appreciation to Mr. Takeshi Minagawa and Mrs. Berlinda Martinez for assisting in the preparation of the manuscript and to Mr. John Viney for technical assistance.

The experimental work discussed in this manuscript was conducted according to the principles enunciated in the "Guide for Laboratory Animal Facilities and Care" prepared by the National Academy of Sciences-National Research Council.

THIS PAGE IS INTENTIONALLY LEFT BLANK.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

THIS PAGE IS INTENTIONALLY LEFT BLANK.

INTRODUCTION

One of the acute problems in clinical biochemistry remains how to detect variations in normal metabolic patterns which may result from a given type of injury, thus enabling one to assess at early time periods which individuals have sustained critical injuries requiring intensive care. The development of automated, high-resolution methods of analysis (reference 13), in which all or many of the constituents of body fluids may be separated and quantitated, appears to offer a new approach for investigating metabolic responses to traumatic injuries and the extent of recovery from such injuries. By use of high-resolution organic acid analyses, propionate accumulations in the bloodstream often were observed to be a feature of critical injuries in animals (reference 6), but it was necessary to resort to loading tests or isotopic labeling of metabolic pools in vivo in order to obtain sufficient sensitivity for studying metabolic responses to nonlethal injuries (references 6 and 7). Various authors have found an increase in early mortality when animals have been exposed to nonlethal levels of ionizing radiation and burn (references 1, 2, 5, 6, and 10). The mechanism appears to result in a more pronounced early shock phase from a given level of thermal injury. This combination of injuries thus might provide an excellent model for investigating metabolic abnormalities occurring at early times following burn injury in experimental animals. Previous studies have shown that $^{14}CO_2$ production in vivo from 1-^{14}C-propionate was significantly depressed by injuries resulting from exposure of rats to airblast, flash burns, or ^{60}Co-gamma irradiation (reference 7), but no studies of combined injuries have been reported.

The initial metabolic response to trauma was termed the "ebb" phase by Cuthbertson (references 4 and 16). This term was used to describe a period of reduced body temperature (small animals) and diminished vitality and metabolism with circulatory deficiency as a central feature. In animals or patients that succumb, the "ebb" phase appears to deepen progressively until a terminal stage of necrobiosis is reached. Individuals recovering from injury appear to progress into a "flow" phase within 24 to 48 hours after injury. The "flow" phase was characterized as a return of body temperature to normal and increased pulse

and respiration along with a marked catabolism of protein. Although it would be useful to estimate the state of recovery from the "ebb" and "flow" phases, no chemical indicators have been found.

A principal change in the concepts of the metabolic responses to trauma is that recent workers have challenged the assumption that decreased heat production in the "ebb" phase was due to anoxia or decreased availability of glucose (references 14 and 15). Although there was agreement that oxygen transport failed during the terminal stages of fatal injury, it was argued that essential organs were oxygenated adequately during the "ebb" phase as indicated by maintenance of the redox state and the phosphorylation state of adenine triphosphate (reference 15). The defect in the "ebb" phase has been ascribed to interruption of the Krebs' cycle at the stage of citric-acid synthesis resulting in decreased heat production. Similar responses were reported in experimental animals exposed to hind-limb ischaemia or thermal injury, but the causes of disruption of oxidative metabolism during the initial metabolic responses to traumatic injuries and the nature of the recovery processes were not explained (references 14, 15, and 16).

The metabolism of organic acids and of propionate, in particular, appears to be the best chemical indicator of the severity of traumatic injuries and the extent of recovery which has been found (references 7 and 8). While the catabolism of labeled compounds in vivo to $^{14}CO_2$ and the elimination of $^{14}CO_2$ through the airways is a complicated process, the metabolism of 1-^{14}C-propionate is considerably simpler than most compounds since only very low pools of propionate appear to exist in vivo, and the transport of propionate into the liver and oxidation in the mitochondria is very rapid (reference 7). As an additional control, it is possible to confirm the significance of reduced rates of $^{14}CO_2$ production by use of 2-^{14}C-propionate. While 100 percent of the ^{14}C from 1-^{14}C-propionate is released as $^{14}CO_2$ in one passage through the Krebs' cycle (reference 7), only 50 percent of the ^{14}C from 2-^{14}C-propionate is released as $^{14}CO_2$ in the second passage through the Krebs' cycle, and 50 percent of the remainder on each additional passage. If there were any metabolic blocks in organic-acid metabolism, 2-^{14}C-propionate injections probably would label the intermediates that accumulate and offer a means of detecting changes in the pattern of organic-acid excretion (reference 7).

Thus, by utilizing highly sensitive radioisotope methods in conjunction with high-resolution analyses of organic-acid excretion patterns, it was hoped to gain insight into the nature of the metabolic problems in traumatic or irradiation injuries and the nature of the recovery mechanisms.

Although radioisotope methods generally may not be applicable to human patients, the progress being made in the synthesis and analysis of compounds containing the stable isotope, ^{13}C, may allow the same approaches to be used in patients with relative safety (reference 12). The synthesis of 1-^{13}C-propionate is relatively simple, and it should be available in the near future.

METHODS

Female, 150-200 g Sprague-Dawley rats were obtained from Bio-Science Laboratories, Oakland, California, and maintained in a constant-temperature room at 25°C and 70 percent relative humidity, with water and Purina Rat Chow supplied ad libitum. When the animals reached a weight of 225-250 g, they were divided randomly into groups of 12 for exposure to thermal, irradiation, or both forms of injury. Irradiation exposure was carried out while animals were restrained in plastic baby-nursing bottles mounted on Masonite panels, with 750 rad of ^{60}Co-gamma irradiation being delivered at a dose rate of 50 rad/minute. After irradiation or sham immobilization for a similar period, the dorsal and lateral surfaces were clipped. Then all animals were put into a state of deep anesthesia with 4 percent Halothane in oxygen, delivered from an anesthesia machine. The thermal and thermal-plus-irradiation groups were exposed to flash burns from a pulsed quartz-lamp source while anesthesized (reference 5). A thermal dose of 9.2 cal/cm^2 over a body area of 88 cm^2 of the lateral and dorsal surfaces corresponded to a full-thickness burn over approximately 30 percent of the body area. At this level of thermal injury, there was minimal penetration of radiant energy into muscle tissue. By omitting air jets which cool the quartz plates over the lamp sources, the output was increased to approximately 10 cal/cm^2, a thermal dose which penetrated through the skin and muscle of the rats but did not penetrate into the abdominal cavity.

Metabolic studies were carried out by injecting animals intraperitoneally with 10 μc of 1- or 2-^{14}C- propionate and maintaining them overnight in glass metabolic cages with water ad libitum. Methods of $^{14}CO_2$ collection and counting were as described previously (reference 7). The 1-^{14}C-propionate (NEC-093H, 20mCi/mmol) and 2-^{14}C-propionate (NEC-051, 5mCi/mmol) were obtained from New England Nuclear Corp., Boston, Massachusetts.

Overnight urine samples were collected in glass metabolic cages in the presence of toluene. No food was given for this period due to difficulties in separating food particles from urine. Samples from six animals were pooled and lyophilized, then dissolved in 1 ml of 10 N H_2SO_4. The solution and

suspended matter were absorbed in 5 g of dried silicic acid (Mallinckrodt No. 2847), and a weighed aliquot of 1/3 to 1/2 of the total sample was added to the top of a 0.9 x 150 cm column containing 25 g of hydrated silicic acid packed in $CHCl_3$ as described previously (references 7, 8, 9, and 11).

After the sample powder was added to the column, a gradient elution cycle (references 7 and 9) was started to separate labeled organic acids. The gradient consisted of four Varigrad chambers connected in series as described by Kesner and Muntwyler (reference 9), and the gradient was pumped through the column at a rate of 100 ml/hr. The gradient consisted of the following solvent mixtures: (a) 150 g of 2 percent v/v 2-methyl-2-butanol in $CHCl_3$; (b) and (c) 150 g of 10 percent v/v 2-methyl-2-butanol in $CHCl_3$; and (d) 150 g of 100 percent 2-methyl-2-butanol. Five-minute fractions were collected directly into 20-ml liquid scintillation vials with a Unifrac Fraction Collector (Savant Instruments, Hicksville, New York). One milliliter of saturated ammonium carbonate solution was added to each vial before use to prevent loss of volatile organic acids. After analysis was complete (approximately 6 hours), the vials were dried overnight at 70°C to remove the organic solvents and excess ammonium carbonate. The residue was dissolved in 1 ml of 0.1 M H_2SO_4 and counted as described previously (reference 7).

Figure 1 shows a chromatogram of standard organic acids run by the above methods. It is seen that these acids are well separated with the exception of lactate and succinate. Since succinate, but not lactate, is an immediate metabolite of propionate in the mitochondria, radioactive peaks eluting in this region probably are succinate. Thus the organic acids involved in the immediate metabolic sequence from propionate are well separated by the gradient developed for this purpose. Extensive tables exist of the relative order in which organic acids elute from silicic acid columns (references 7, 8, 9, and 11), so that, when unknown peaks appear, a good guess can be made of their identity and proper standards then run as tentative proof. The recovery of organic acids by this procedure ranges from better than 75 percent for $\mu\mu$mole quantities of labeled organic acids to better than 95 percent for labeled organic acids chromatographed in the presence of 0.1 μmole of unlabeled carrier. A major factor affecting the recovery of organic acids is that the sample must be acidified to a pH of 2 or below, as the partition chromatography is based on separation of the undissociated acids.

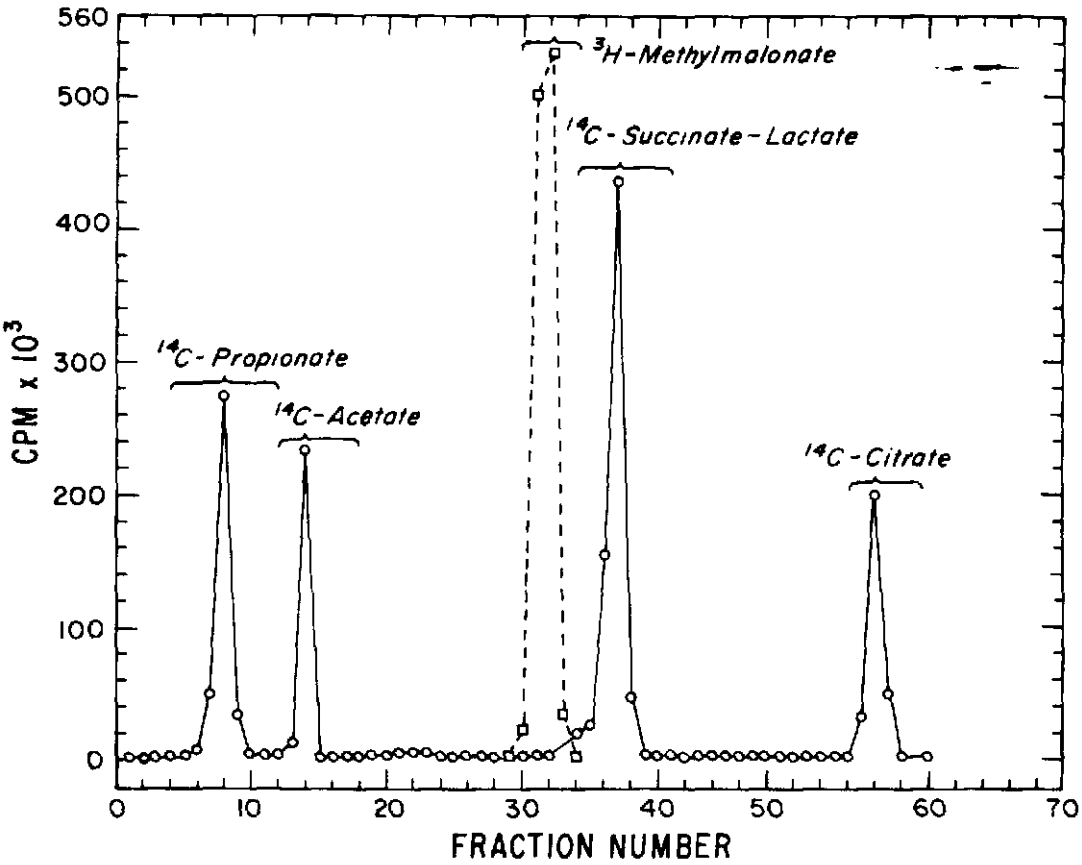

Figure 1.--Separation of standard ^{14}C-organic acids by partition chromatography. [For details, see methods section. Sample contained about 0.02 μCi of each of the following acids: succinic acid-1, 4-^{14}C (20 mCi/mmol); LD-lactic acid-1-^{14}C (38.3 mCi/mmol); acetic acid-1-^{14}C (57 mCi/mmol); propionic acid-1-^{14}C (20 mCi/mmol); citric acid-1, 5-^{14}C (22 mCi/mmol); and 2-methyl-^{3}H-malonate (50 mCi/mmol).]

RESULTS

A comparison of the effects of flash burns and gamma irradiation, singly and in combination, on mortality in rats is shown in table 1. There was no detectable early mortality in rats exposed to 750 rad, and the 30-day mortality was less than 10 percent. The standard flash-burn injury resulted in a mortality of 20 percent or less in the first 7 days after exposure, and only an occasional rat died at later time periods. Conversely, rats exposed to irradiation plus flash burns exhibited approximately 90 percent mortality after 7 days, and none were alive 14 days after exposure. These results show that exposure of rats to combinations of injuries may greatly increase mortality compared to rats exposed to single injuries.

A comparison of the effects of single or combined injuries on the initial rate of 1-^{14}C-propionate oxidation in vivo is shown in figure 2. All injuries significantly reduced the rate of propionate oxidation when the animals were tested 4 hours after exposure. However, animals exposed to both flash burns and irradiation showed an impaired recovery from the initial injury and remained outside the 2 standard deviation line from controls at all time intervals until death. Animals exposed to single injuries exhibited a considerable degree of recovery by 24 hours post exposure.

These results indicate that the initial rate of 1-^{14}C- propionate oxidation in vivo is a useful screening method for determining the times after injury that oxidative metabolism is depressed and the extent of recovery therefrom.

As a confirmatory test, we have investigated the metabolism of 2-^{14}C-propionate, which is oxidized at a slower rate than 1-^{14}C-propionate. If any significant metabolic blocks existed in organic-acid metabolism, any intermediates that accumulated probably would be labeled and excreted in the urine in increased amounts.

When the rate of 2-^{14}C-propionate oxidation to $^{14}CO_2$ was measured in rats exposed to single or combined injuries, a greater depression was noted in animals exposed to combined injury (figure 3). An additive effect of flash burns and gamma irradiation was found at 4 hours after injury compared to the reduction

TABLE 1. --Survival times of rats exposed to ^{60}Co-gamma irradiation, thermal injuries, or both*

No. Animals Surviving	Days Post Exposure								
	0	1	2	3	4	5	6	7	14
Fasting Controls	6	6	6	6	6	6	6	6	6
Irradiation only	48	48	48	48	48	48	48	48	48
Thermal injury only	48	41	39	39	39	39	38	38	38
Irradiation plus thermal injury	48	25	20	12	12	9	6	4	0

* Thermal injuries of 9.2 cal/cm^2 delivered to an area of 88 cm^2 and (or) 750 rads of ^{60}Co-gamma irradiation (midline air dose). No immediate lethality was observed within the initial 4 hours with any of the single or multiple injuries.

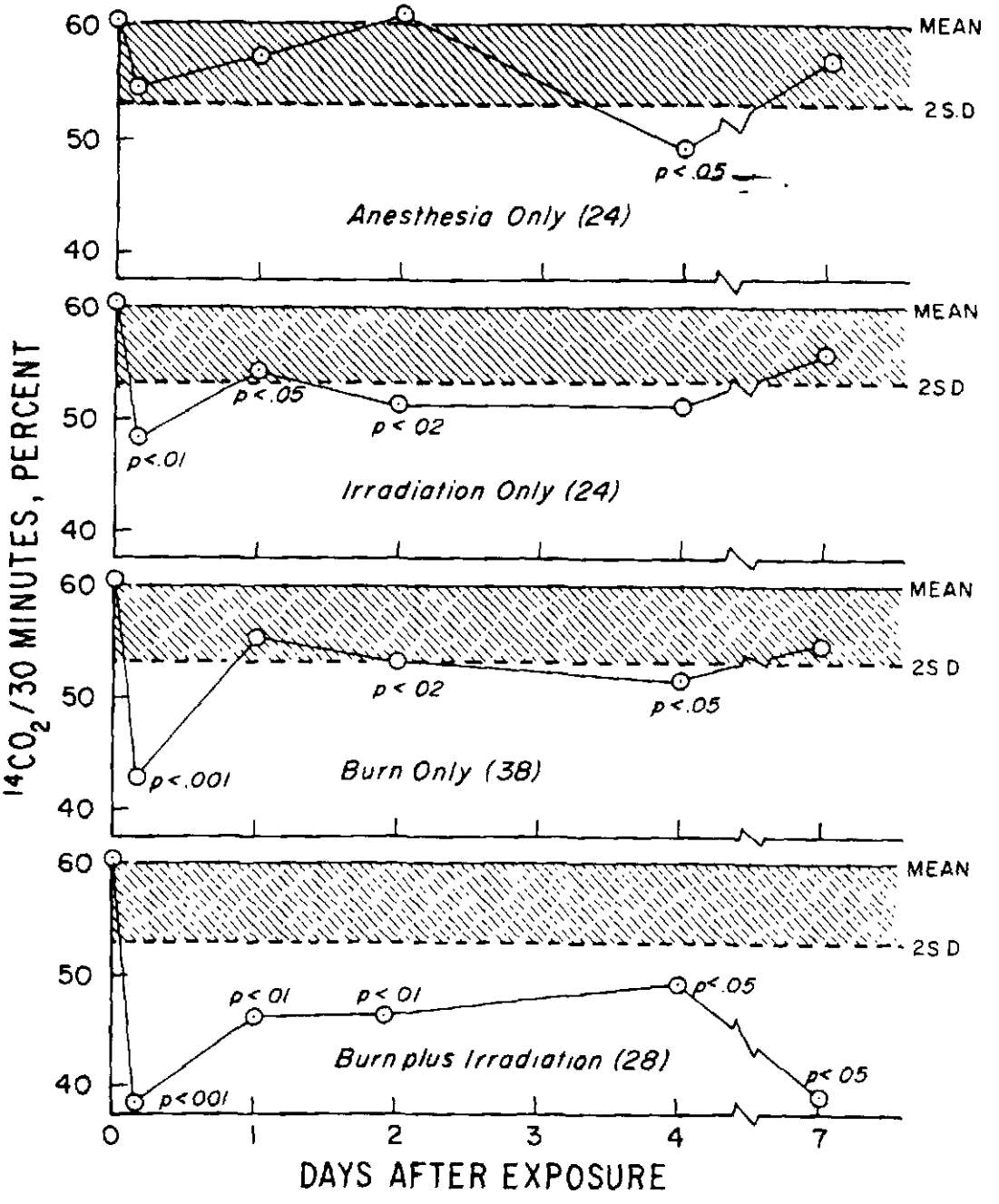

Figure 2. --Effects of single and combined injury on 1-^{14}C-propionate oxidation in vivo. [Rats were injected IP with 10 μCi of 1-^{14}C-propionate and the $^{14}CO_2$ collected for 30 minutes and counted by liquid scintillation. The test was repeated at the indicated times after exposure to injury. The 2 S. D. line is two standard deviations from pre-exposure values, and the p values represent the significance compared to control values by Students' t-test. The number in parenthesis indicates the number of animals in each group.]

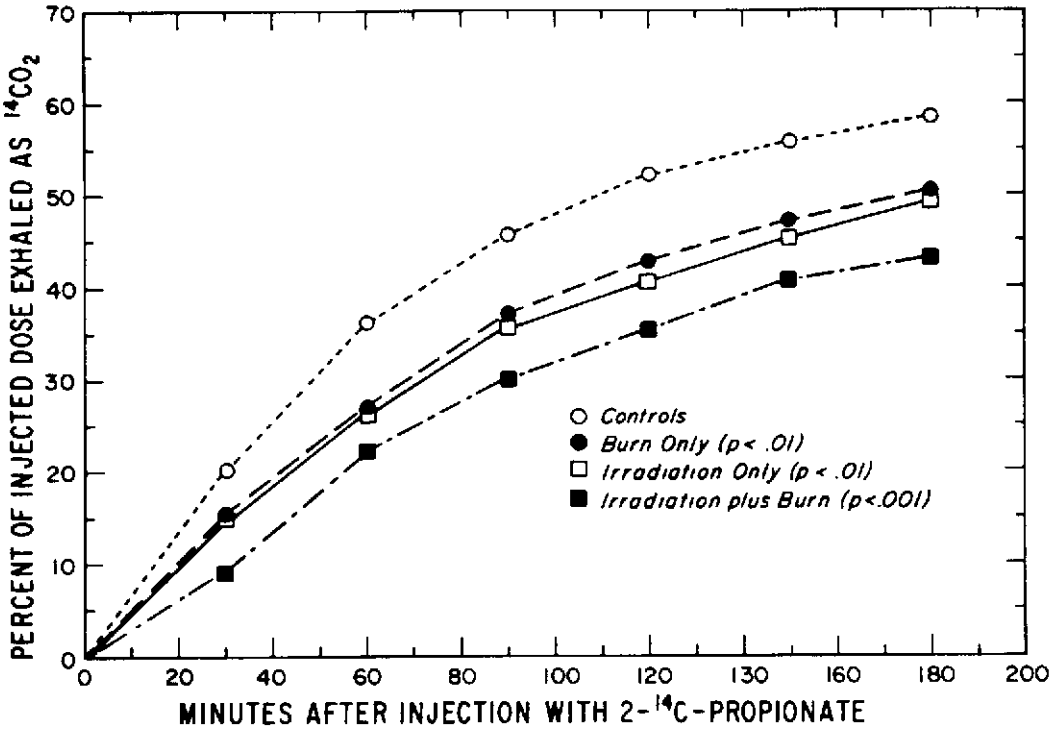

Figure 3. --Effects of single and combined injuries on 2-^{14}C-propionate oxidation in vivo. [Conditions were as described in Figure 2 except 10 μCi of 2-^{14}C-propionate was used. All animals were tested 4 hours after exposure to the indicated injury, and $^{14}CO_2$ samples collected at 30-minute intervals for 3 hours. (n = 6 in each group.)]

in propionate oxidation rate due to either injury alone. Since the initial rate of $^{14}CO_2$ production was reduced and the effect persisted over the entire period of observation, this is evidence that the utilization of propionate through the Krebs' cycle was altered. If this effect of thermal and/or gamma irradiation were simply due to slow absorption or slow release of $^{14}CO_2$, one would expect to see a lesser effect on 2-^{14}C-propionate oxidation than on 1-^{14}C- propionate because of a slower rate of oxidation.

When urine samples were collected overnight from rats after injection with 2-^{14}C-propionate, marked differences occurred in the patterns of organic-acid excretion before and after injury. Approximately 0.5 percent of the injected dose was excreted in the urine of control animals; approximately 75 percent of this radioactivity was in the form of citric acid, and smaller amounts were associated with propionate and methylmalonate peaks (figure 4). The remainder of the radioactivity eluted from the silicic acid column was not associated with noticeable peaks and was assumed to be miscellaneous compounds only slightly soluble in $CHCl_3$-2-methyl-2-butanol mixtures.

After exposure to injuries, the excretion of labeled organic acids decreased markedly in all instances. A typical example is shown in figure 5, where rats exposed to flash burns exhibited only a slight citric-acid excretion and small amounts of propionate and methylmalonate when tested 24 hours after injury. Animals that survived experimental injuries tended to return to pre-exposure excretion patterns in 2 to 3 weeks, but secondary peaks were frequently noted in the interim.

The variations in ^{14}C-citrate excretion after propionate injection by animals at various times after injury are summarized in figure 6. Either irradiation or thermal injury reduced ^{14}C-citrate excretion, as did exposure of animals to both injuries. Irradiation and flash burns alone did not result in as marked a decrease in ^{14}C-citrate excretion, and a considerable degree of recovery was noted by 48 hours to 1 week after injury. There was no sign of recovery in the combined-injury animals, the ^{14}C-citrate excretion remaining about 1/20 of control values from injury until death. Not only was the total ^{14}C-citrate excretion decreased, but the ^{14}C-citrate excretion as a percentage of total counts recovered from silicic-acid columns decreased. In the case of combined-injury animals,

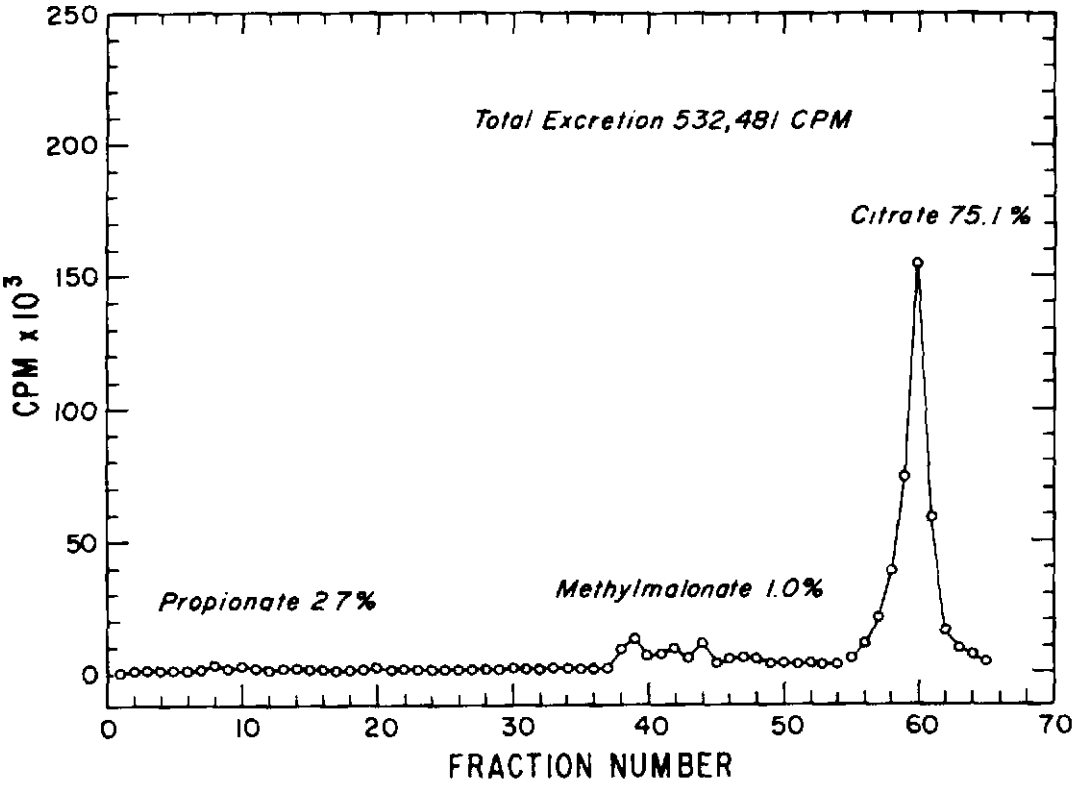

Figure 4. --Organic acid excretion patterns of control rats. [Rats were injected IP with 10 μc of 2-^{14}C-propionate and urine collected in glass metabolic cages for 24 hours with water given ad libitum. Urine samples were concentrated and analyzed chromatographically for organic acids as described in methods section. The total excretion refers to total counts recovered from urine samples by partition chromatography. The percent (%) figures refer to the CPM recovered in each peak/total excretion.]

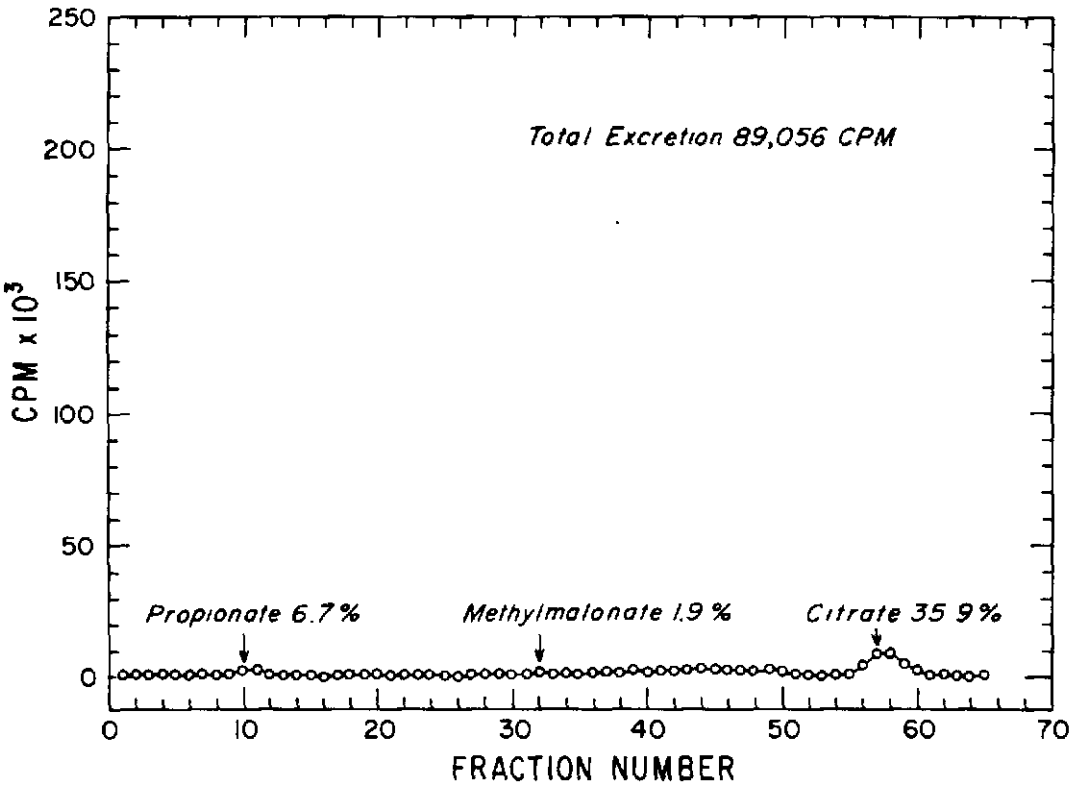

Figure 5. --Organic acid excretion patterns of rats exposed to thermal injury. [Conditions were as described in figure 4. Rats were tested 24 hours after injury. (n = 6.)]

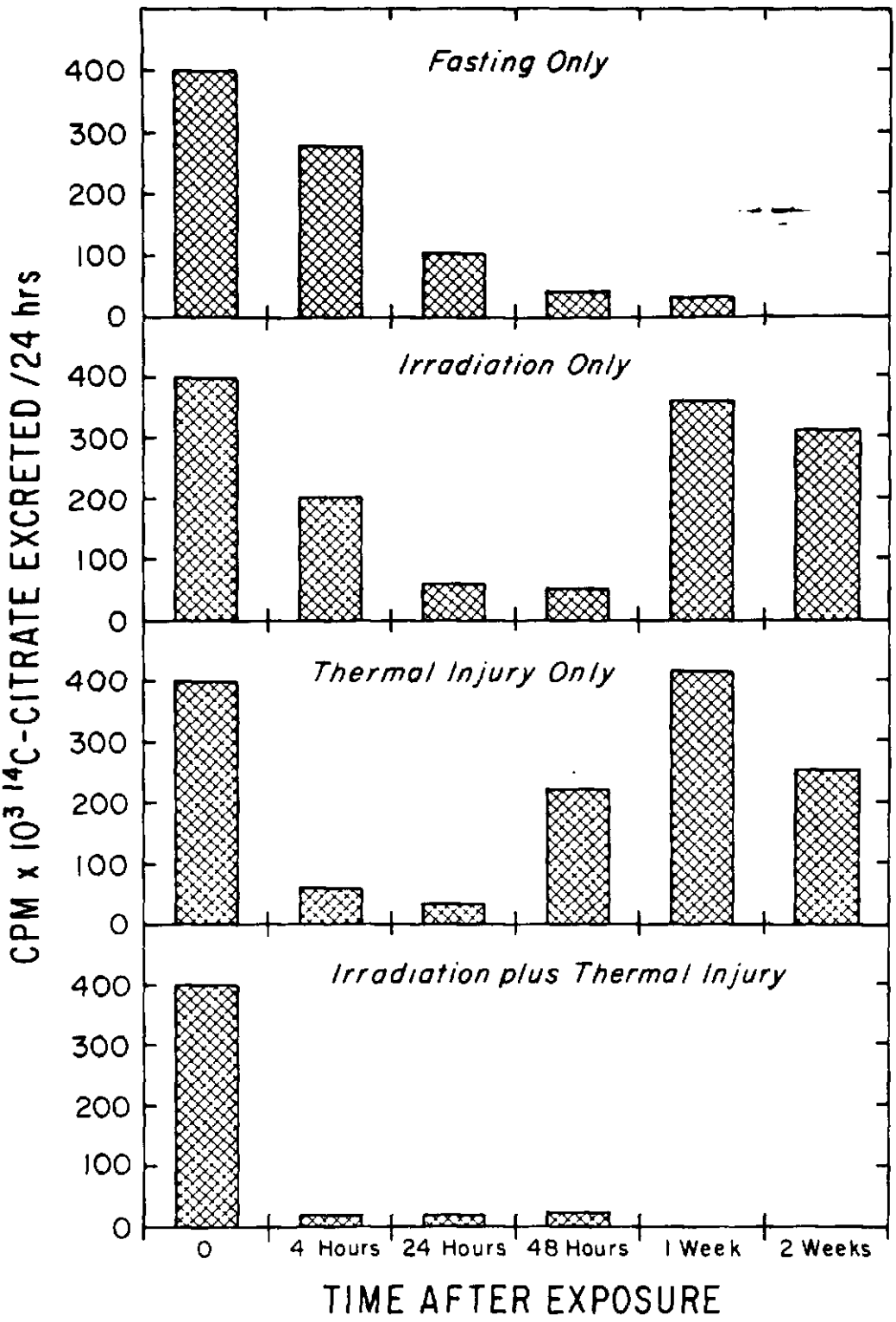

Figure 6. --^{14}C-Citrate excretion after single or combined injury. (Conditions were as described in figure 4.) [The total citrate excretion following propionate injection is given in total CPM/24 hrs. (n = 6 in each group.)]

^{14}C-citrate was only 17 percent of the total eluted counts at 24 hours after injury; while in fasted, irradiated, or burned animals 24 hours after injury, ^{14}C-citrate was 55 percent, 33.5 percent, and 35.9 percent of the total recovered counts, respectively.

The levels of ^{14}C-citrate excretion were found to be more markedly changed by exposure of animals to combined injury than the total citrate excretion. Citrate concentration in urine samples from control animals was 5.18 ± 1.78 μmoles/ml, whereas combined-injury animals exhibited a urinary citrate level of 1.64 ± 1.04 μmoles/ml in the first 24 hours after injury. Thus the citrate pool in liver mitochondria which becomes labeled in vivo and escapes into the urine showed approximately a twentyfold change following combined injury, while the citrate derived from the total cellular pool only showed a threefold change in the same time period.

Although the total radioactivity excreted as propionate showed slight changes, when calculated as a percentage of total cpm recovered from silicic acid columns, there was a steady increase associated with the deterioration of combined-injury animals (figure 7). Irradiation or thermal injury appeared to result in transient changes in the percentage of organic acids excreted as propionate, but recovery occurred rapidly.

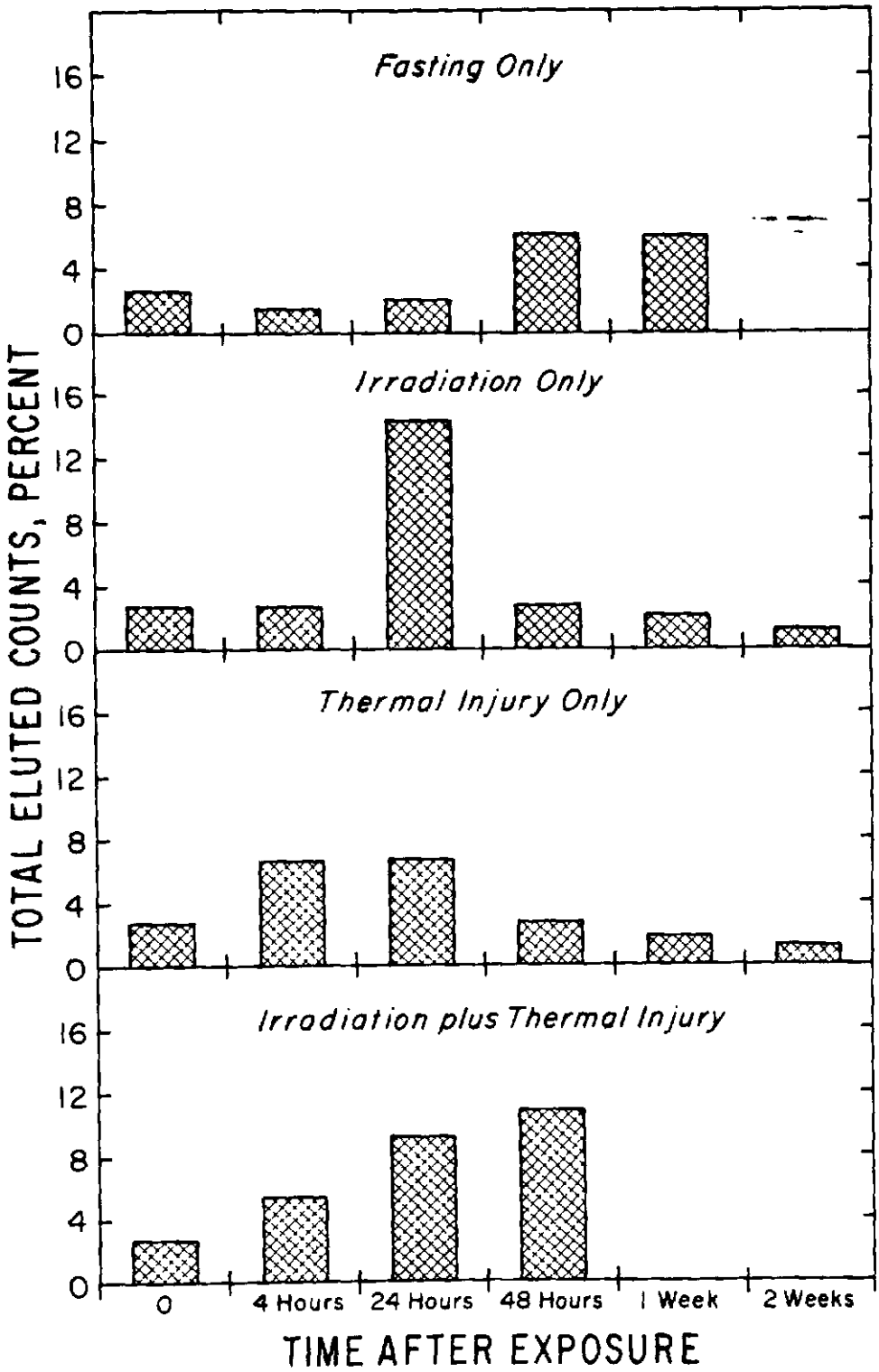

Figure 7.--^{14}C-Propionate excretion after single or combined injury. [Conditions were as described in figure 4. The total propionate excretion is given as percentage (%) of total excretion.]

DISCUSSION

The association between a persistently depressed rate of propionate oxidation and impending lethality in rats exposed to thermal injury plus gamma irradiation is similar to that which has been observed in rats exposed to single injuries of greater magnitude (reference 7). However, both the time to death and the extent of inhibition of propionate oxidation appear to be different in animals exposed to combined injury. The data presented here suggest that irradiation interferes with recovery from the "ebb" or shocklike phase following thermal injury.

The "ebb" phase was characterized as a reduced Krebs' cycle activity at the site of citrate synthesis and a reduced rate of oxidation of ^{14}C-organic acids by the Krebs' cycle (reference 15). Our results show that the "ebb" phase can be monitored best by the rate of oxidation of 1- or 2-labeled propionate because the pathway of propionate intake into the Krebs' cycle is unidirectional and not branched as it is for other acids like pyruvate (reference 7). Since propionate appears to be rapidly transported into the mitochondria and incorporated into Krebs' cycle intermediates (reference 18), it is reasonable to assume that the nature of the labeled organic acids which escape and are excreted in the urine might be related to the size of the pool of each acid in the mitochondria and the facility with which they become labeled from propionate in vivo.

Thus, the greatly decreased excretion of ^{14}C-citrate by rats following injury is an indication that the citrate pools of liver mitochondria have become low. Since no other organic acids are excreted in exaggerated amounts at early times after injury, it is difficult to interpret the defect as one of a blockage of the Krebs' cycle as proposed by Threlfall (reference 15). Rather, a more likely explanation is that the "ebb" phase is characterized by a deficiency of Krebs' cycle intermediates in the mitochondria, since a major factor affecting the rate of citric-acid formation in rat-liver mitochondria appears to be the availability of oxalacetate (references 17 and 19). Supplementation with Krebs' cycle intermediates may be a useful adjunct to therapy in traumatic injuries, as such administrations have been reported to reduce the mortality from hemorrhagic shock in animals (reference 3).

Insufficient regeneration of Krebs' cycle intermediates could account for a deficiency of them in the liver mitochondria after injuries (reference 17). When oxalacetate is used in the Krebs' cycle during energy production, it is regenerated by passage through the cycle and functions catalytically. However, when oxalacetate is utilized in synthetic activities, such as glucose production by the liver, it is not regenerated but must be replenished from other sources such as amino acids (reference 17).

Thus, the results reported here suggest that the "ebb" phase following injury may be characterized by a deficiency of Krebs' cycle intermediates in the mitochondria. The net effects would be a decreased oxidative metabolism and heat production due to decreased citrate levels in the mitochondria (reference 15). Recovery from the "ebb" phase into the "flow" phase could be associated with development of an increased capacity for mobilization of reserve materials such as proteins as a source of Krebs' cycle intermediates. Since mobilization of amino acids for energy production involves the induction of de novo enzyme synthesis, an early effect of irradiation could result from the effects of genetic damage on RNA synthesis.

REFERENCES

1. Alpen, E. L., and Sheline, G. E. "The Combined Effects of Thermal Burns and Whole Body X-Irradiation on Survival Time and Mortality." Ann Surg 140, pp. 113-118, 1954.
2. Baker, D. G., and Valeriote, F. A. "The Effect of X-Irradiation and Thermal Burn in the Intestinal Mucosa." Can J Phys Pharm 46, pp. 533-536, 1968.
3. Chick, W. L., Weiner, R., Cascarano, J., et al. "Influence of Krebs Cycle Intermediates on Survival from Hemorrhagic Shock." Am J Phys 215, pp. 1107-1110, 1968.
4. Cuthbertson, D. P., and Tilstone, W. J. "Metabolic Changes Following Different Types of Injuries and Their Relation to Diet and Environmental Temperature." In Schildt, B., and Thoren, L. (Editors): Proceedings of the Symposium on Combined Injury and Shock. Stockholm, Försvarets Forskninganstalt, 1968, pp. 99-114.
5. Damon, E. G. Personal communication.
6. Henderson, T. R., and Jones, R. K. "Serum Organic Acids in Sheep Exposed to Neutron-Gamma Irradiation, Air Blast or Both." Clin Chem 16, pp. 697-701, 1970.
7. Henderson, T. R., and Jones, R. K. "Effects of Irradiation or Traumatic Injuries on Propionate Metabolism in Rats." Clin Chem 17, pp. 810-817, 1971.
8. Henderson, T. R., Viney, J. F., and Jones, R. K. "Induction of Rat Liver Tyrosine Aminotransferase by Therma and Irradiation Injuries." Fed Proc 31, p. 865, 1972.
9. Kesner, L., and Muntwyler, E. "Separation of Citric Acid Cycle and Related Compounds by Partition Column Chromatography." In Lowenstein J. M. (Editor): Methods in Enzymology Vol XIII. New York, Academic Press, pp. 415-430, 1969.
10. Levenson, S. M., Einheber, A., and Crowley, L. V. "Some Effects of Whole Body X-Irradiation and Thermal Injury." In Artz, C. P. (Editor): Research in Burns. Philadelphia, F. A. Davis Co., pp. 143-148, 1962.

11. Rosenvear, J. W., Pfaff, K. J., and Moffitt, E. A. "High-Resolution Chromatographic System for Measuring Organic Acids in Biological Samples." Clin Chem 17, pp. 721-730, 1971.

12. Schreiber, R. E. (Editor): "ICONS at LASL." Los Alamos Scientific Laboratory Report LA-4759-MS, 1971.

13. Scott, C. D., and Melville, R. S. "Introduction to Proceedings of Second Annual Symposium on Automated, High-Resolution Analyses in the Clinical Laboratory." Clin Chem 16, pp. 623-624, 1970.

14. Stoner, H. B. "The Acute Effects of Trauma on Heat Production." In Porter, R., and Knight, J. (Editors): Energy Metabolism in Trauma. London, J & A Churchill, pp. 1-7, 1970.

15. Threlfall, C. J. "Intermediary Carbohydrate Metabolism in Injured Rat Liver in Relation to Heat Production." Ibid, pp. 127-144.

16. Tilstone, W. J., and Cuthbertson, D. P. "The Protein Component of the Disturbance of Energy Metabolism in Trauma." Ibid, pp. 43-53.

17. Utter, M. F. "Metabolic Roles of Oxalacetate." In Lowenstein, J. M. (Editor): Citric Acid Cycle Control and Compartmentation. New York, Marcel Dekker, pp. 249-296, 1969.

18. Weideman, M. J., Hems, R., Williams, D. L., et al. "Gluconeogenesis from Propionate in Kidney and Liver of the Vitamin B_{12}-Deficient Rat." Biochem J 117, p. 177, 1970.

19. Williamson, J. R., Scholz, R., and Browning, E. T. "Control Mechanisms of Gluconeogenesis and Ketogenesis. Interactions Between Fatty Acid Oxidation and the Citric Acid Cycle in Perfused Rat Liver." J Biol Chem 244, pp. 4617-4627, 1969.

DISTRIBUTION LIST

COPIES

DEPARTMENT OF DEFENSE

1 DIRECTOR, ARMED FORCES INSTITUTE OF PATHOLOGY, WALTER REED ARMY MEDICAL CENTER, 14TH STREET & ALASKA AVENUE, WASHINGTON, D.C. 20012

5 DIRECTOR, ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE, DEFENSE NUCLEAR AGENCY, NATIONAL NAVAL MEDICAL CENTER, BETHESDA, MARYLAND 20014
ATTN: COR

1 ASSISTANT TO THE SECRETARY OF DEFENSE, ATOMIC ENERGY, WASHINGTON, D.C. 20301
ATTN: DOCUMENT CONTROL

1 DEFENSE CIVIL PREPAREDNESS AGENCY, WASHINGTON, D.C. 20301
ATTN: RE, ASST. DIR. RESEARCH, MR. WALMER E. STROPE

12 DEFENSE DOCUMENTATION CENTER, CAMERON STATION, ALEXANDRIA, VIRGINIA 22314
ATTN: TC

5 DIRECTOR, DEFENSE NUCLEAR AGENCY, WASHINGTON, D.C. 20305
ATTN: DDST
ATTN: APSI (ARCHIVES)
ATTN: TECHNICAL LIBRARY, APTL (2 CYS)
ATTN: RAAE

2 COMMANDER, FIELD COMMAND, DEFENSE NUCLEAR AGENCY, KIRTLAND AFB, NEW MEXICO 87115
ATTN: TECHNICAL LIBRARY, FCTA-A
ATTN: FCTD, TEST DIRECTORATE

1 DIRECTOR, WEAPONS SYSTEMS EVALUATION GROUP, ODDR&E OFFICE, SECRETARY OF DEFENSE, 400 ARMY-NAVY DRIVE, WASHINGTON, D.C. 20305
ATTN: CAPT. DONALD E. MCCOY, USN

COPIES

DEPARTMENT OF THE ARMY

1 COMMANDER, ABERDEEN RESEARCH AND DEVELOPMENT CENTER, ABERDEEN PROVING GROUND, MARYLAND 21005
ATTN: MR. JULIUS J. MESZAROS

1 COMMANDANT, ARMY MEDICAL FIELD SERVICE SCHOOL, BROOKE ARMY MEDICAL CENTER, FORT SAM HOUSTON, TEXAS 78234

1 CHIEF, ARMY RESEARCH OFFICE, LIFE SCIENCES DIVISION, 3045 COLUMBIA PIKE, ARLINGTON, VIRGINIA 22210

1 CHIEF OF RESEARCH & DEVELOPMENT, DEPARTMENT OF THE ARMY, WASHINGTON, D.C. 20310
ATTN: NCB DIVISION, LTC C. R. SYKES

1 DEPARTMENT OF THE ARMY, HARRY DIAMOND LABORATORIES, WASHINGTON, D.C. 20438
ATTN: AMXDO-NP

1 COMMANDING GENERAL, MEDICAL RESEARCH AND DEVELOPMENT COMMAND, WASHINGTON, D.C. 20314
ATTN: NBC SCIENCES DIVISION

1 THE SURGEON GENERAL, DEPARTMENT OF THE ARMY, WASHINGTON, D.C. 20360

1 U.S. ARMY COMBAT DEVELOPMENTS COMMAND, NUCLEAR AGENCY, FORT BLISS, TEXAS 79916
ATTN: CDINS-E

1 CHIEF, U.S. ARMY COMBAT DEVELOPMENTS COMMAND, MEDICAL SERVICE AGENCY, FORT SAM HOUSTON, TEXAS 78234

1 CHIEF, U.S. ARMY COMBAT DEVELOPMENTS COMMAND, CHEMICAL-BIOLOGICAL-RADIOLOGICAL AGENCY, FORT MCCLELLAN, ALABAMA 36205
ATTN: CBR

1 DIRECTOR, WALTER REED ARMY INSTITUTE OF RESEARCH, WALTER REED ARMY MEDICAL CENTER, WASHINGTON, D.C. 20012
ATTN: DOCUMENTS LIBRARY

COPIES

DEPARTMENT OF THE NAVY

1 CHIEF, BUREAU OF MEDICINE AND SURGERY, NAVY DEPARTMENT, WASHINGTON, D.C. 20390

1 CHIEF OF NAVAL OPERATIONS, NAVY DEPARTMENT, WASHINGTON WASHINGTON, D.C. 20350
ATTN: OP-985F

2 CHIEF OF NAVAL RESEARCH, DEPARTMENT OF THE NAVY, ARLINGTON, VIRGINIA 22217
ATTN: CODE 402, MR. AUBREY W. PRYCE, DEP. DIR. RESEARCH
ATTN: CODE 418, DR. THOMAS P. QUINN, DIR. FIELD PROJ. PRO.

1 COMMANDING OFFICER, NAVAL AEROSPACE MEDICAL INSTITUTE, NAVAL AEROSPACE MEDICAL CENTER, PENSACOLA, FLORIDA 32512

2 COMMANDER, NAVAL AIR SYSTEMS COMMAND, RM 522, JEFFER JEFFERSON PLAZA NO. 1, WASHINGTON, D.C. 20360
ATTN: AIR 5381, SATELLITE COMM/ASTRO-DEFENSE BRANCH
ATTN: AIR-350, ORDNANCE ADMINISTRATOR

1 COMMANDING OFFICER, NAVAL MEDICAL RESEARCH INSTITUTE, NATIONAL NAVAL MEDICAL CENTER, BETHESDA, MARYLAND 20014

4 COMMANDER, NAVAL ORDNANCE LABORATORY, SILVER SPRING, MARYLAND 20910
ATTN: CODE 210, DR. WAYNE W. SCANLON
ATTN: CODE 213, MR. WILLIAM L. DERKSEN
ATTN: CODE 121, NAVY NUCLEAR PROGRAMS OFFICE
ATTN: CODE 121, DR. O. M. MEREDITH

1 DIRECTOR, NAVAL RESEARCH LABORATORY, WASHINGTON, D.C. 20390
ATTN: CODE 2027, TECHNICAL LIBRARY

1 COMMANDER, NAVAL SHIP RESEARCH AND DEVELOPMENT CENTER, BETHESDA, MARYLAND 20034
ATTN: CODE L42.3, LIBRARY

1 COMMANDING OFFICER, NAVAL SUBMARINE MEDICAL CENTER, NAVAL SUBMARINE BASE-NEW LONDON, GROTON, CONNECTICUT 06340

COPIES

DEPARTMENT OF THE NAVY (Continued)

1 COMMANDING OFFICER, NAVAL WEAPONS EVALUATION FACILITY, KIRTLAND AIR FORCE BASE, ALBUQUERQUE, NEW MEXICO 87117
ATTN: DOCUMENT CONTROL

1 COMMANDER, NAVAL WEAPONS LABORATORY, DAHLGREN, VIRGINIA 22448
ATTN: CODE TIEC

DEPARTMENT OF THE AIR FORCE

1 COMMANDER, AEROSPACE MEDICAL DIVISION, AFSC, BROOKS AFB, TEXAS 78235
ATTN: COL WILLIAM R. GODDEN, AMRB

1 COMMANDER, AF SPECIAL WEAPONS CENTER, AFSC, KIRTLAND AFB, NEW MEXICO 87117
ATTN: SWTSX, SURVIVABILITY/VULNERABILITY BRANCH

1 AF WEAPONS LABORATORY, AFSC, KIRTLAND AFB, NEW MEXICO 87117
ATTN: DOUL, TECHNICAL LIBRARY

1 HEADQUARTERS, AIR FORCE SYSTEMS COMMAND, ANDREWS AFB, WASHINGTON, D.C. 20331
ATTN: SGI

2 CHIEF OF STAFF, U.S. AIR FORCE, WASHINGTON, D.C. 20330
ATTN: PDPS (SCI. & TECH. DIV., DIR. OF DEV. & ACQ.)
ATTN: RDQPN (S/V & NUCLEAR PROGRAMS DIVISION)

1 DIRECTOR OF NUCLEAR SAFETY, THE INSPECTOR GENERAL, KIRTLAND AFB, NEW MEXICO 87117

1 THE SURGEON GENERAL, DEPARTMENT OF THE AIR FORCE, JAMES FORRESTAL BUILDING, WASHINGTON, D.C. 20314

1 USAF MEDICAL SERVICE SCHOOL, SHEPPARD AFB, TEXAS 76311

COPIES

DEPARTMENT OF THE AIR FORCE (Continued)

1 USAF RADIOLOGICAL HEALTH LABORATORY, AFLC, WRIGHT-PATTERSON AFB, OHIO 45433

3 USAF SCHOOL OF AEROSPACE MEDICINE, AFSC, BROOKS AFB, TEXAS 78235
ATTN: RA, CHIEF, RADIOBIOLOGY DIV.
ATTN: RAT
ATTN: EDAD

1 6570TH AEROSPACE MEDICAL RESEARCH LABS., AFSC, WRIGHT-PATTERSON AFB, OHIO 45433

ATOMIC ENERGY COMMISSION

1 ASSISTANT GENERAL MANAGER FOR MILITARY APPLICATION, U.S. ATOMIC ENERGY COMMISSION, WASHINGTON, D.C. 20545
ATTN: DOCUMENT CONTROL FOR R&D BRANCH

1 BATTELLE MEMORIAL INSTITUTE, PACIFIC NORTHWEST LABORATORY, P.O. BOX 999, RICHLAND, WASHINGTON 99352
ATTN: DR. K. H. LARSON

1 BROOKHAVEN NATIONAL LABORATORY, P.O. BOX 150, UPTON, LONG ISLAND, NEW YORK 11973
ATTN: RESEARCH LIBRARY

1 LOS ALAMOS SCIENTIFIC LABORATORY, P.O. BOX 1663, LOS ALAMOS, NEW MEXICO 87544

1 MANAGER, TECHNICAL INFORMATION CENTER, U.S. ATOMIC ENERGY COMMISSION, P.O. BOX 62, OAK RIDGE, TENNESSEE 37830
ATTN: DOCUMENT CONTROL

2 U.S. ATOMIC ENERGY COMMISSION, DIVISION OF HEADQUARTERS SERVICES, LIBRARY BRANCH G-049, WASHINGTON, D.C. 20545
ATTN: DOCUMENT CONTROL FOR REPORTS SECTION
ATTN: DOC. CONTROL (BIO. & MED. DIV.), H.D. BRUNER

1 U.S. ATOMIC ENERGY COMMISSION, NEW YORK OPERATIONS OFFICE, 376 HUDSON STREET, NEW YORK, NEW YORK 10014
ATTN: DOCUMENT CONTROL FOR DR. J.H. HARLEY, DIR. HASL

COPIES

ATOMIC ENERGY COMMISSION (Continued)

1 UNION CARBIDE CORPORATION, OAK RIDGE NATIONAL LABORATORY, P.O. BOX X, OAK RIDGE, TENNESSEE 37830
ATTN: DOCUMENT CONTROL FOR J. AUXIER, HEALTH PHY. DEPT.

1 UNION CARBIDE CORPORATION, NUCLEAR DIVISION, P.O. BOX Y, OAK RIDGE, TENNESSEE 37830
ATTN: DOC CONT-ATOMIC/MOL. PROC. INFO. CTR-C.F. BARNETT

1 UNIVERSITY OF CALIFORNIA, LAWRENCE LIVERMORE LABORATORY, TECHNICAL INFORMATION DIVISION, P.O. BOX 808, LIVERMORE, CALIFORNIA 94550
ATTN: TECHNICAL LIBRARY

OTHER GOVERNMENT

1 NASA SCIENTIFIC AND TECHNICAL INFORMATION FACILITY, P.O. BOX 33, COLLEGE PARK, MARYLAND 20740
ATTN: ACQUISITIONS AND INPUT BRANCH

1 NATIONAL LIBRARY OF MEDICINE, ACCESSIONS BRANCH, 8600 WISCONSIN AVENUE, BETHESDA, MARYLAND 20014

DEPARTMENT OF DEFENSE CONTRACTORS

1 CINCINNATI GENERAL HOSPITAL, RADIOISOTOPE LABORATORY, CINCINNATI, OHIO 45229
ATTN: DR. EUGENE L. SAENGER, DIR., RADIOISOTOPE LAB.

1 GENERAL ELECTRIC COMPANY, TEMPO-CENTER FOR ADVANCED STUDIES, 816 STATE STREET (P.O. DRAWER QQ), SANTA BARBARA, CALIFORNIA 93102
ATTN: DASIAC

104 LOVELACE FOUNDATION FOR MEDICAL EDUCATION AND RES., 5200 GIBSON BOULEVARD, S.E., ALBUQUERQUE, NEW MEXICO 87108
ATTN: ASST. DIR. OF RESEARCH, DR. ROBERT K. JONES (100 CYS)
ATTN: PRESIDENT-DIRECTOR, DR. CLAYTON S. WHITE
ATTN: HEAD DEPT. COMP. ENVIR. BIOL., DR. DONALD RICHMOND
ATTN: ASSOCIATE SCIENTIST, DR. EDWARD G. DAMON
ATTN: ASSOCIATE SCIENTIST, DR. E. ROYCE FLETCHER

UNCLASSIFIED
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1 ORIGINATING ACTIVITY (Corporate author)
Lovelace Foundation for Medical Education and Research, 5200 Gibson Boulevard SE, Albuquerque, New Mexico 87108

2a. REPORT SECURITY CLASSIFICATION
UNCLASSIFIED

2b GROUP

3 REPORT TITLE
Organic Acids as Metabolic Indicators. The Metabolism of ^{14}C-Propionate in Rats Exposed to Irradiation and Thermal Injuries

4 DESCRIPTIVE NOTES (Type of report and inclusive dates)
Topical Report

5 AUTHOR(S) (First name, middle initial, last name)
Thomas R. Henderson and Robert K. Jones

6 REPORT DATE	7a. TOTAL NO. OF PAGES	7b NO. OF REFS
February 1973	38	19

8a CONTRACT OR GRANT NO	9a ORIGINATOR'S REPORT NUMBER(S)
DA-49-146-XZ-359 b PROJECT NO NWER: QAXM c Task and Subtask: A112 d Work Unit: 03	DNA 3002T 9b OTHER REPORT NO(S) (Any other numbers that may be assigned this report)

10 DISTRIBUTION STATEMENT
Approved for public release; distribution unlimited.

11 SUPPLEMENTARY NOTES	12 SPONSORING MILITARY ACTIVITY
	Director Defense Nuclear Agency Washington, D.C. 20305

13 ABSTRACT

^{14}C-propionate utilization was found to be a useful in vivo indicator of the severity of burn or irradiation injuries and the extent of recovery from such injuries. Flash burns (9.2 cal/cm^2, 30 percent of body area) or gamma irradiation (750 rad) produced a significantly reduced rate of $^{14}CO_2$ production from 1-^{14}C-propionate during the initial shock phase following exposure. Recovery from nonlethal injuries was associated with recovery of ability to oxidize propionate within 1 to 2 weeks. In the case of animals exposed to irradiation plus flash burns, a greater initial decrease in propionate oxidation was noted and recovery did not occur. The persistence of decreased rates of propionate oxidation often was correlated with early mortality.

It was concluded that a deficiency of Krebs' cycle intermediates was a significant characteristic of the initial metabolic state or "ebb" phase following injuries and that biochemical events sensitive to irradiation were involved in recovery. Measurements of the rate and pathway of propionate utilization appeared to be a useful physiological monitor for estimating the extent of recovery from thermal burns.

DD FORM 1 NOV 65 1473

UNCLASSIFIED
Security Classification

14 KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Propionate metabolism						
Citrate metabolism						
Burns						
Irradiation						
Combined injury						
Organic acid metabolic						
Metabolic indicators						