AFWL TR
65-112

EFFECTS OF RADIATION ON SOME SERUM ENZYMES AND TRACE ELEMENTS IN LARGE ANIMALS

William S. Riggsby
1st Lt USAF

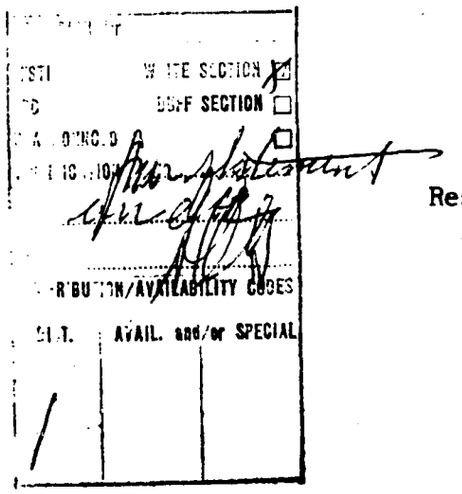
Norman D. Jones
Captain USAF VC

William R. Godden
Major USAF VC

TECHNICAL REPORT NO. AFWL-TR-65-112

January 1966

AIR FORCE WEAPONS LABORATORY
Research and Technology Division
Air Force Systems Command
Kirtland Air Force Base
New Mexico



Research and Technology Division
AIR FORCE WEAPONS LABORATORY
Air Force Systems Command
Kirtland Air Force Base
New Mexico

AFWL-TR-65-112

(U) EFFECTS OF RADIATION ON SOME SERUM ENZYMES AND TRACE ELEMENTS IN LARGE ANIMALS

William S. Riggsby
1st Lt USAF

Norman D. Jones
Captain USAF VC

William R. Godden
Major USAF VC

TECHNICAL REPORT NO. AFWL-TR-65-112

FOREWORD

This report is the result of the combined effort of the Air Force Weapons Laboratory and the Omaha Veterans Administration Hospital, Omaha, Nebraska, under Contract AF 29(601)-62-5217 and amendments.

The research was performed under Program Element 6.24.05.12.4, Project 7801, Task 780104. Inclusive dates of research were 1 April 1962 to 7 June 1965. The report was submitted in October, 1965, by the AFWL Project Officer, Captain Norman D. Jones, (WLRB).

The authors wish to acknowledge the staff of the Laboratory of Nuclear Medicine and Biology and the Radioisotope Service of the Omaha Veterans Administration Hospital under the guidance of Richard E. Ogborn, MD, Chief, for their efforts in performing the tests and processing the myriad data. The authors also wish to thank Merton A. Quaife, Captain, USAF MC, who was the USAF Project Officer and contractor monitor during the actual period of experimentation.

Animal experimentations reported herein were conducted in accordance with the "Principles of Laboratory Animal Care" of the National Society for Medical Research.

This technical report has been reviewed and is approved.

NORMAN D. JONES
Captain, USAF VC
Project Officer

MAX M. NOLD
Colonel, USAF VC
Chief, Biophysics Branch

WILLIAM H. STEPHENS
Colonel, USAF
Chief, Research Division

ABSTRACT

This report summarizes the results of determinations of serum zinc, copper, malic dehydrogenase (MDH), lactic dehydrogenase (LDH), glutamic-oxalacetic transaminase (SGOT), and glutamic-pyruvic transaminase (SGPT) in large mammals following various types and doses of radiation. The principal subjects were mature sheep although beagle dogs and "miniature" swine were also used. The radiation sources were a cobalt-60 teletherapy unit, a 250-kvp X-ray therapy unit, and a Godiva II pulsed fission-spectrum neutron reactor. Results indicate that the quantity and type of irradiation received by these animals cannot be determined from these parameters.

This page intentionally left blank

CONTENTS

ILLUSTRATIONS

TABLES

This page intentionally left blank

SECTION I

INTRODUCTION

Although extensive information is available concerning the response to radiation of micro-organisms and small animals, relatively little is known about these effects in animals with a body mass comparable to that of man. Because of the difference in size between man and, for example, the rat, the radiative energy disposition pattern with respect to the configuration of the body is quite different. Consequently one cannot necessarily extrapolate to man results obtained with small animals or bacteria. While the basic mechanism may be the same, the depth-dose relationships and the tissue interactions must vary from case to case. For these reasons, it was felt that useful information bearing on the probable radiation responses of man could be obtained from experiments using mammals of comparable size.

The experiments reported here were undertaken as a part of a program to attempt to establish a quantitative correlation between dose absorbed by large mammals (principally sheep), and subsequent measurable alterations in the physiological state of the organism.

The present research was based on several previously reported experiments. It has been reported that patients undergoing radiation therapy showed increased serum zinc levels (reference 1). There have been many reports of altered serum enzyme levels in various mammalian species, including man, as a result of irradiation (references 2 through 13). Zinc is known to be required by a number of enzyme systems, including some of those in which radiation-induced alterations of activity have been observed (references 14 through 17). In other studies, end products of these enzyme reactions have been observed to vary in both humans and experimental animals after irradiation (references 18 through 21). It has been postulated that, from an investigation of serum zinc levels following irradiation, a pattern of response might evolve, and that this parameter might be useful as a biological indicator of exposure to ionizing radiation.

A pilot study with guinea pigs as the experimental subjects was previously carried out to determine whether there was a dose-dependent alteration of serum zinc levels following exposure to ionizing radiation. The principal results of

this study are shown in figure 1, where the observed serum zinc levels are plotted as a function of time post irradiation for several dose levels. Note the large variation among the subjects receiving the same dose, as indicated by the error bars. This study was limited by several technical difficulties. Because guinea pigs are small, complete exsanguination was required for the duplicate determination of serum zinc levels; thus, a new group of subjects was used for each point in the time-course of the experiment. The time dependence of the response of an individual could not be followed, nor could post-irradiation values for an individual be compared with a pre-irradiation value for that individual. Furthermore, the guinea pig does not satisfy the requirement that the experimental subjects have a body mass comparable to that of man.

The investigation into the alteration of serum zinc levels was then begun on three larger mammalian species -- sheep, beagle dogs, and "miniature" swine. The relatively large blood volume of these animals allowed multiple pre- and post-exposure values to be determined. The involvement of zinc in dehydrogenase reactions led to the inclusion of malic and lactic dehydrogenase as parameters to be measured also. Serum copper and two transaminases were included in the study because of interest by the professional/technical personnel at the Omaha Veterans Administration Hospital (OVAH). Finally, a more detailed study of the serum levels of these enzymes was carried out in sheep following exposure to either pulsed fission-spectrum neutrons or 250-kvp X rays. This report will be concerned primarily with the enzyme studies, although the results on serum zinc and copper will be introduced as appropriate.

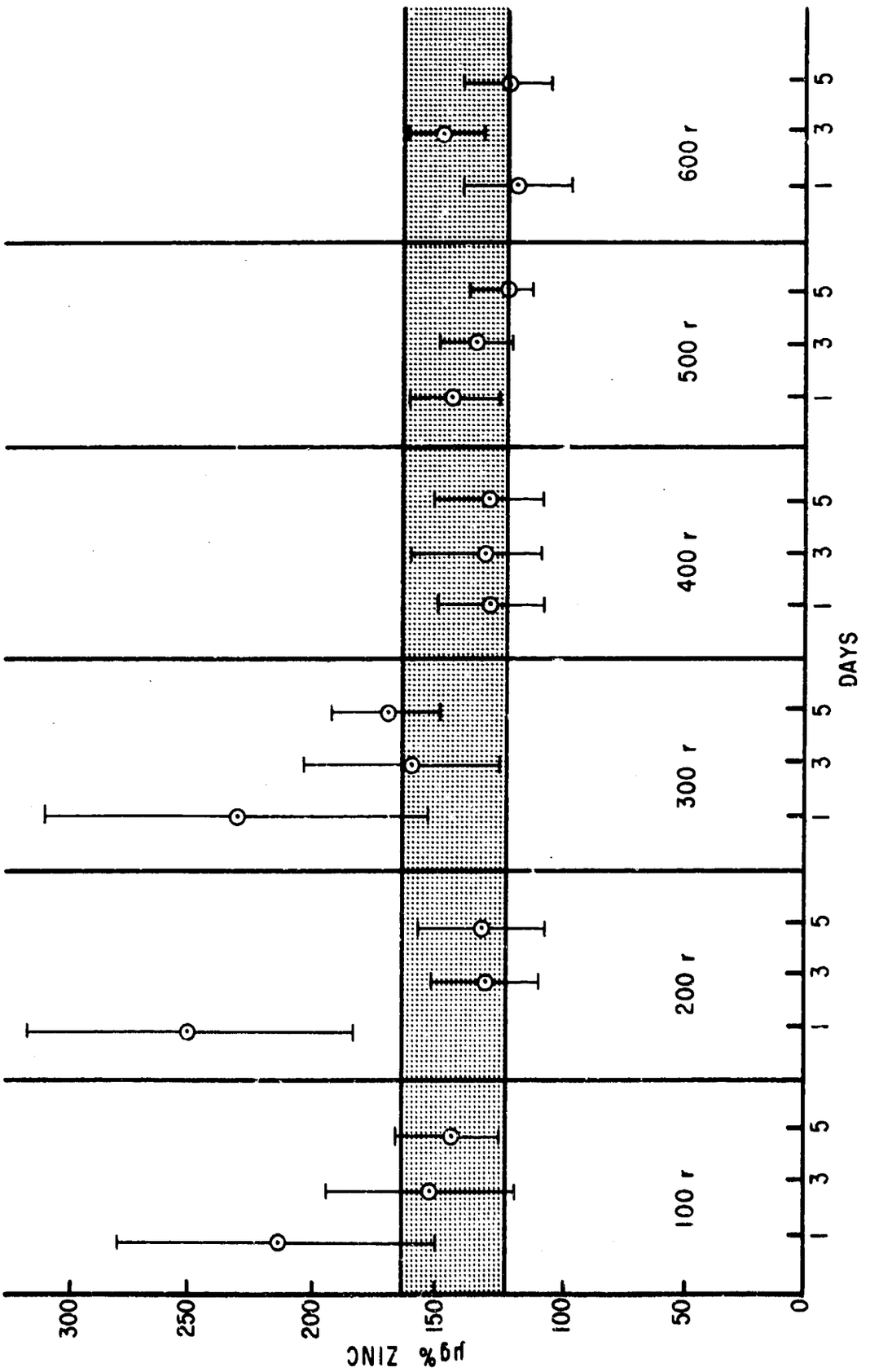


Figure 1. Serum Zinc Levels in Guinea Pigs after Co^{60} Gamma Irradiation

SECTION II

METHODS AND MATERIALS

1. Animal Handling and Sampling

Purebred beagle dogs (male and female) were held and sampled at OVAH. Blood was drawn from the jugular vein with 21-gauge, 1.5-inch needles.

Genetically controlled miniature swine (barrows) were held and sampled at OVAH. Blood was drawn from the superior vena cava with 18-gauge, 4-inch needles (reference 22).

Two populations of sexually mature female sheep were used. Blood samples were taken by the same method as that used for beagles. Sheep for Co^{60} experiments were held and sampled at OVAH. Those for X-ray and neutron studies were held, irradiated, and sampled at the Air Force Weapons Laboratory (AFWL). The method of handling is reported in AFWL-TR-65-109 (reference 23). Samples taken at AFWL were frozen and shipped to OVAH for processing.

2. Irradiation Procedures

A Picker Co^{60} teletherapy unit with a 500-curie source was used as the gamma-ray source. Dogs were irradiated in lucite boxes and were exposed at either 5.0 R/min or 4.4 R/min midline air dose (MAD). Swine were irradiated in heavy wooden cages, and were exposed at 3.2 R/min MAD. Sheep were irradiated in plywood boxes, and were exposed at 1.2 R/min MAD. Exposure rates were measured with a Siemens-Reinigemerke R meter.

The X-ray source was a standard 250-kvp (HVL -1.56 mm Cu) therapeutic unit modified to produce a wide X-ray field. The X rays were filtered through 1 mm Al and 0.56 mm Cu. The midline air dose delivered was 10 R/min, and the energy delivered was equivalent to 97 kev. The sheep were held and exposed in wooden crates. Dosimetry was carried out by radiophotoluminescent glass rod and ionization chamber techniques described elsewhere (reference 24).

The neutron source was the Sandia Pulsed Reactor Facility (SPRF), which produces fission-spectrum neutrons in bursts of 50 microseconds. These sheep were also irradiated in wooden crates. The dose can be controlled both by the reactor controls and by the positioning of the sheep. Dosimetry was carried out at AFWL (reference 25) and included activation and fission-foil analysis for neutrons, and glass rods for associated gamma radiation. The neutron-to-gamma ratio was 5:1.

3. Chemical Techniques

Zinc content of the samples was determined by the method described in the Appendix.

Copper content of samples was determined by the method of Rice (reference 26). Lactic dehydrogenase was determined by the method of Wroblewski and LaDue (reference 27). Malic dehydrogenase was determined by the method of Ochoa (reference 28). Transaminases were determined by the method of Reitman and Frankel (reference 29) using kits purchased from Uni-Tech Manufacturing Company, Panorama City, California. As a check, similar kits purchased from Hyland Laboratories, Los Angeles, California were cross-calibrated with the Uni-Tech kits, and were found to give essentially the same results.

SECTION III

RESULTS

1. General Comments

In this section some representative results will be presented briefly. Although detailed comment will be reserved for the discussion, it should be pointed out here that the results are largely negative in terms of the objectives of this study. In view of the overall character of the data, only a small part of them are presented here. Because of the similarity of the results obtained in all species, this presentation will be confined largely to the data obtained from sheep experiments.

Reference is made to several data population classes which are defined as follows:

a. Baseline: Values obtained from the entire population before the eginning of irradiation exposures.

b. Controls: Animals subjected to treatment identical to the irradiated subjects except that they were not exposed to the radiation source. These animals were crated, transported, and bled so as to induce in them as nearly as possible the same basic physiological state as the irradiated subjects.

c. Irradiated Subjects: These animals were exposed to the radiation environment specified in each case. These subjects are further subdivided operationally into two subclasses:

(1) Lethals: Those which died as an apparent result of the radiation received.

(2) Survivors: Those which did not.

2. Serum Zinc Levels

Serum zinc levels in sheep at various times after irradiation with various doses of Co^{60} gamma radiation are shown in figure 2 and table I. The baseline value was 76 ± 12, while the aggregate of the controls gave a value of 95 ± 25. Six sets of controls, one associated with each of the irradiated sets, gave values as follows over the entire period of the experiment:

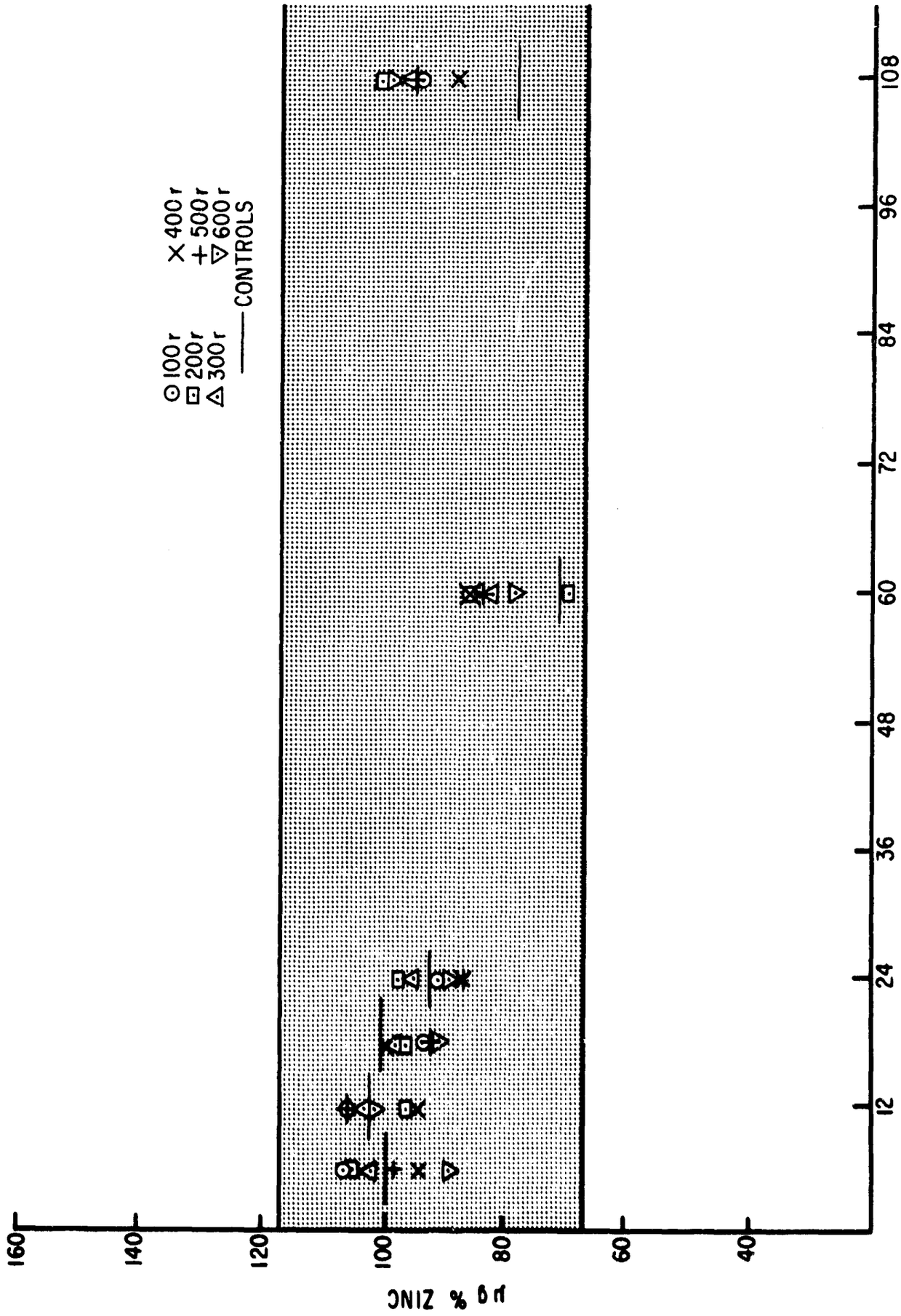


Figure 2. Serum Zinc Levels in Sheep Exposed to X rays

Table I

SERUM ZINC LEVELS OF CONTROL SHEEP FROM THE DIFFERENT IRRADIATION GROUPS

Irradiation Group	µg % Zn Average Std Dev
100 R	87 ± 23
200 R	100 ± 22
300 R	90 ± 23
400 R	87 ± 21
500 R	105 ± 34
600 R	86 ± 21

Units are µg % (micrograms of Zn per 100 ml plasma).

In figure 2, the shaded area indicates the aggregate average of the controls and one standard deviation above and below. The points indicate the averages for each irradiation level and time, as well as the corresponding average of all controls at that time. Note that all averages of exposed groups fall well within one standard deviation of the control mean.

Because of the spread in values observed, and because one objective of the study was to establish possible dose-response patterns relevant to man, a study was done at OVAH on Zn levels in human plasma. Eighty-four subjects were used, yielding an average of 92 µg percent and a standard deviation of 17 percent. Several subjects in this study were sampled repeatedly over a period of weeks to determine the variations within an individual. The ranges (in µg percent) for these individuals are given in table II. In some of these subjects, differences of nearly a factor of 2 were observed as a function of time.

3. Serum Copper Levels

The results of the serum copper analyses are qualitatively similar to the serum zinc results, except that the observed variations are similar. Generally, both subjects and control groups differed from the baseline values by less than 10 percent. These results, it should be noted, were obtained with dogs and swine, since serum copper determinations in sheep were not attempted.

4. Serum Enzyme Levels

In this section we will deal with the results obtained by high-dose irradiation of sheep by X rays and neutrons. The enzymes selected for the study were serum glutamic-oxalacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), and the zinc-requiring dehydrogenases of lactic acid (LDH) and

Table II

RANGES OF ZINC LEVEL IN HUMAN PLASMA

Subject	Range	Maximum difference
	ug %	ug %
1	56-85	29
2	70-102	32
3	63-116	53
4	67-114	47
5	64-102	38
6	57-111	54
7	72-123	51
8	74-104	30
9	74-104	30
10	89-117	28
11	71-112	41
12	70-127	57
13	73-112	39

malic acid (MDH). To present a cross section of the results, we include here the results obtained from one enzyme at four dose levels, and for all four enzymes at one dose level. These data for animals exposed to fission-spectrum neutrons are given in tables IIIa to IXa and for 250-kvp X rays in table IIIb to IXb. The response parameter shown, x, is the mean percent of the baseline value. The standard deviation for each group is given. Immediately after the value for each group is the number of subjects used to obtain that value.

These data have been chosen as representing the most significant radiation-dependent changes in enzyme levels. The enzymes other than SGOT, at doses less than 518 R, tend to show fewer differences between controls and subjects. Moreover, although no detailed quantitative tests have been carried out, the other results appear to have even less statistical significance than those shown in tables III to IX.

Table III

SGOT LEVELS IN SHEEP EXPOSED TO 518 RADS

Mean percent of baseline value

Time after exposure	Controls	Lethals	Survivors	Lethals & survivors
		a. Neutrons		
3 hr	132±28 (4)	115±14 (4)	124±22 (4)	119±18 (8)
6 hr	90± 9 (4)	101±15 (4)	123±16 (4)	112±18 (8)
1 d	68±29 (4)	82±38 (4)	105±21 (4)	93±31 (8)
2 d	110±18 (4)	128± 9 (4)	182±71 (4)	154±55 (8)
3 d	156±34 (4	147±29 (4)	201±94 (4)	173±70 (8)
10 d	121±33 (4)	112±10 (2)	156±69 (4)	141±58 (6)
17 d	95±22 (4)		112±42 (4)	112±42 (4)
24 d	207±88 (4)		252±42 (4)	252±42 (4)
31 d	133±47 (4)		183±41 (4)	183±41 (4)
45 d	109±29 (4)		140±21 (4)	140±21 (4)
52 d	89±19 (4)		123±42 (4)	123±42 (4)
59 d	99± 4 (4)		150±41 (4)	150±41 (4)
		b. X rays		
3 hr	138±40 (4)	142±26 (8)		
6 hr	136±44 (4)	160±29 (8)		
3 d	106±24 (4)	118±13 (8)		
10 d	103±18 (4)	101±23 (8)		
17 d	109±13 (4)	104±21 (8)		

Table IV

SGPT LEVELS IN SHEEP EXPOSED TO 518 RAD

Mean Percent of Baseline Value

Time after exposure	Controls	Lethals	Survivors	Lethals & survivors
		a. Neutrons		
3 hr	73±50 (4)	47±10 (4)	73±11 (4)	60±17 (8)
6 hr	56± 8 (4)	24±16 (4)	75±22 (4)	50±33 (8)
1 d	111±22 (4)	81±34 (4)	145±86 (4)	113±70 (8)
2 d	55±24 (4)	86±40 (4)	83±37 (4)	85±36 (8)
3 d	193±40 (4)	112±23 (4)	203±33 (4)	158±56 (8)
10 d	287±136(4)	182± 2 (2)	413±246(4)	337±225(6)
17 d	83±10 (4)		84±25 (4)	84±25 (4)
24 d	271±112(4)		246±89 (4)	246±89 (4)
31 d	83±34 (4)		112±37 (4)	112±37 (4)
45 d	31±10 (4)		46±42 (4)	46±42 (4)
52 d	68±14 (4)		63±14 (4)	63±14 (4)
59 d	84±20 (4)		89± 9 (4)	89± 9 (4)
		b. X rays		
3 hr	121±68 (4)	101±27 (8)		
6 hr	124±64 (4)	124±32 (8)		
3 d	127±25 (4)	126±21 (8)		
10 d	116±83 (4)	103±78 (8)		
17 d	161±61 (4)	139±56 (8)		

Table V

LDH LEVELS IN SHEEP EXPOSED TO 518 RAD

Mean Percent of Baseline Value

Time after exposure	Controls	Lethals	Survivors	Lethals & survivors
		a. Neutrons		
3 hr	83±14 (4)	83±46 (4)	102±17 (4)	92±34 (8)
6 hr	103±35 (4)	134±29 (4)	120±31 (4)	127±28 (8)
1 d	84±11 (4)	178±15 (4)	180±44 (4)	179±30 (8)
2 d	173±38 (4)	207±31 (4)	236±58 (4)	221±46 (8)
3 d	126±43 (4)	154±37 (4)	166± 5 (4)	160±25 (8)
10 d	150±39 (4)	228±11 (2)	219±91 (4)	222±71 (6)
17 d	201±39 (4)		261±79 (4)	261±79 (4)
24 d	91±32 (4)		136±40 (4)	136±40 (4)
31 d	92±19 (4)		150±45 (4)	150±45 (4)
45 d	65±20 (4)		88±16 (4)	88±16 (4)
52 d	79±29 (4)		89±11 (4)	89±11 (4)
59 d	68±16 (4)		80±16 (4)	80±16 (4)
		b. X rays		
3 hr	104±14 (4)	116±17 (8)		
6 hr	104± 5 (4)	129±19 (8)		
3 d	96±16 (4)	118±24 (8)		
10 d	118±19 (4)	97±17 (8)		
17 d	96± 7 (4)	89±11 (8)		

Table VI

MDH LEVELS IN SHEEP EXPOSED TO 518 RAD

Mean Percent of Baseline Value

Time after exposure	Controls	Lethals	Survivors	Lethals & survivors
		a. Neutrons		
3 hr	121±31 (4)	94±53 (4)	101±21 (4)	98±37 (8)
6 hr	84±25 (4)	123±38 (4)	107±24 (4)	115±31 (8)
1 d	91±11 (4)	146± 5 (4)	132±38 (4)	138±26 (8)
2 d	234±37 (4)	208±64 (4)	280±65 (4)	244±71 (8)
3 d	140±85 (4)	160±30 (4)	160±62 (4)	160±45 (8)
10 d	182±64 (4)	256±32 (2)	206±65 (4)	222±58 (6)
17 d	291±36 (4)		233±54 (4)	233±54 (4)
24 d	158±75 (4)		153±39 (4)	153±39 (4)
31 d	116±49 (4)		218±92 (4)	218±92 (4)
45 d	72±20 (4)		76±20 (4)	76±20 (4)
52 d	62± 8 (4)		91±24 (4)	91±24 (4)
59 d	72±26 (4)		76±14 (4)	76±14 (4)
		b. X rays		
3 hr	114±11 (4)	119±19 (8)		
6 hr	109± 9 (4)	137±32 (8)		
3 d	103±15 (4)	108±30 (8)		
10 d	116±11 (4)	112±23 (8)		
17 d	95±18 (4)	68±10 (8)		

Table VII

SGOT LEVELS IN SHEEP EXPOSED TO 476 RAD

Mean Percent of Baseline Value

Time after exposure	Controls	Lethals	Survivors	Lethals & survivors
		a. Neutrons		
3 hr	78±17 (4)	85±18 (2)	91±26 (6)	89±23 (8)
6 hr	74±20 (4)	76± 2 (2)	102±33 (6)	96±31 (8)
1 d	115± 9 (4)	131±25 (2)	149±47 (6)	144±42 (8)
2 d	66± 6 (4)	63± 3 (2)	75±15 (6)	72±14 (8)
3 d	103±18 (4)	100±25 (2)	141±32 (6)	131±35 (8)
10 d	95±17 (4)	100±32 (2)	171±95 (6)	153±88 (8)
17 d	150±47 (4)	206 (1)	203±127 (6)	204±113 (7)
31 d	28± 6 (4)		40±23 (6)	40±23 (6)
38 d	89±12 (4)		151±15 (6)	151±15 (6)
45 d	83±16 (4)		122±18 (6)	122±18 (6)
52 d	86±14 (4)		137±25 (6)	137±25 (6)
59 d	101±23 (4)		150±25 (6)	150±25 (6)
		b. X rays		
3 hr	139±81 (4)	127±29 (7)	128 (1)	127±27 (8)
6 hr	151±100 (4)	137±32 (7)	130 (1)	136±30 (8)
3 d	89±24 (4)	105±18 (7)	224 (1)	120±45 (8)
10 d	105±29 (4)	109±22 (7)	108 (1)	109±20 (8)
17 d	66±14 (4)	65± 9 (6)	55 (1)	63± 9 (7)
24 d	70±19 (2)	60 (1)	66 (1)	63± 4 (2)
31 d	70±12 (2)	114 (1)	49 (1)	81+46 (2)
38 d	60±13 (2)		49 (1)	49 (1)
45 d	69±19 (2)		49 (1)	49 (1)
52 d	52± 7 (2)		43 (1)	43 (1)
59 d	61± 5 (2)		49 (1)	49 (1)

Table VIII

SGOT LEVELS IN SHEEP EXPOSED TO 359 RAD

Mean Percent of Baseline Values

Time after exposure	Controls	Lethals	Survivors	Lethal & survivors
		a. Neutrons		
3 hr	73±27 (4)		59±18 (8)	
6 hr	65±14 (4)		66±13 (8)	
1 d	109±48 (4)		110±31 (8)	
2 d	188±82 (4)		191±78 (8)	
3 d	133±45 (4)		190±63 (8)	
10 d	88±44 (4)		79±24 (8)	
17 d	99±36 (4)		108±48 (8)	
24 d	117±47 (4)		108±42 (8)	
31 d	94±42 (4)		99±30 (8)	
38 d	108±32 (4)		133±35 (8)	
45 d	71±19 (4)		89±39 (8)	
52 d	115±42 (4)		165±51 (8)	
59 d	115±33 (4)		168±70 (8)	
		b. X rays		
3 hr	66±24 (4)	80±23 (2)	70±10 (6)	73±13 (8)
6 hr	66±25 (4)	81±38 (2)	75± 5 (6)	76±15 (8)
1 d	69± 7 (4)	60±18 (2)	73±22 (6)	70±20 (8)
2 d	68± 8 (4)	92 (1)	68±28 (6)	72±27 (7)
3 d	64± 7 (4)	84± 3 (2)	68±26 (6)	72±24 (8)
10 d	52±11 (4)	64±37 (2)	55±15 (6)	57±19 (8)
17 d	51± 8 (4)	47±18 (2)	51± 6 (6)	50± 9 (8)
24 d	56±10 (4)	64± 1 (2)	54± 6 (6)	56± 7 (8)
31 d	59±15 (4)		56±11 (6)	56±11 (6)
38 d	61±25 (4)		57± 7 (6)	57± 7 (6)
45 d	52±14 (4)		54±10 (6)	54±10 (6)
52 d	70±17 (4)		76±12 (6)	76±12 (6)
59 d	53±35 (4)		49±28 (6)	49±28 (6)

Table IX

SGOT LEVELS IN SHEEP EXPOSED TO 218 RAD

Mean Percent of Baseline Values

Time after exposure	Controls	Lethals	Survivors	Lethals & Survivors
		a. Neutrons		
3 hr	125± 5 (4)		138±70 (8)	
6 hr	116± 6 (4)		133±47 (8)	
1 d	166±66 (4)		141±43 (8)	
2 d	29± 2 (4)		43±16 (8)	
3 d	80±21 (4)		85±29 (8)	
17 d	78± 9 (4)		64±18 (8)	
24 d	87± 5 (4)		77±21 (8)	
31 d	98± 3 (4)		90±26 (8)	
38 d	86± 8 (4)		76±17 (8)	
45 d	73± 5 (4)		70±13 (8)	
52 d	115±11 (4)		103±27 (8)	
59 d	73± 7 (4)		77±30 (8)	
		b. X rays		
3 hr	74±21 (4)		74±14 (8)	
6 hr	76±17 (4)		77±14 (8)	
1 d	66±20 (4)		72±17 (8)	
2 d	66±16 (4)		73±14 (8)	
3 d	72±24 (4)		77±20 (8)	
10 d	95±18 (4)		89±22 (8)	
17 d	61±42 (4)		67±51 (8)	
24 d	55±25 (4)		56±21 (8)	
31 d	105±28 (4)		108±28 (8)	
38 d	121±38 (4)		120±19 (8)	
45 d	106±22 (4)		95±30 (8)	
52 d	81±12 (4)		93±25 (8)	
59 d	93±16 (4)		109±13 (8)	

SECTION IV

DISCUSSION

1. General Comments

While the results presented in the last section do, in some instances seem to indicate a radiation-induced alteration in blood trace element and enzyme levels, the alterations observed are not of the type hoped for when the study was initiated. Because of the relatively small number of subjects in each experimental group, and the large fluctuations within the groups and in one individual as a function of time, it is impossible to draw general conclusions as to the effects of radiation on the sera parameters involved. In particular, conventional biostatistical methods are not applicable to such small populations, and even the calculations of the standard deviation must be regarded as a rough indication of the dispersion of the data rather than as a reliable statistical parameter. Several suggestive results did emerge, however, and these will be discussed in paragraphs 2 and 3.

2. Trace Element Levels

In the serum zinc levels there is at least a suggestion of a radiation-induced alteration in some instances. It would be difficult to maintain, for example, that the serum zinc elevation in guinea pigs shown in figure 1 is a matter of chance. The relatively large number of subjects in the pilot study lends credence to these results. Moreover, for certain post-irradiation times in sheep, the mean serum zinc levels for every irradiated group lies to one side or the other of the control mean (figure 2). Similar results were noted in swine and dogs.

As noted earlier, no significant differences were observed between the serum copper levels of controls and irradiated subjects, either in dogs or in swine.

Even though serum zinc results at one time or another appear to be significant, they are of no practical value in in vivo dosimetry. In figure 2, for example, although all the irradiated subject means at 108 hours lie above the control mean, they are still within one standard deviation of the control mean as measured over the entire course of the experiment and indicated by the cross-hatched area. That is to say, the elevation at 108 hours is significant only

when compared with controls sampled at the same time, but not when compared with a control-population mean taken over a period of time. In any practical situation, one would not have available the appropriate control mean at the corresponding time; and even if such a mean were attainable the variation from individual to individual about the mean would introduce a further uncertainty. We shall return to the question of individual variation later.

3. Serum Enzyme Levels

In subjects exposed to 250-kvp X rays, no alteration in serum enzyme levels was found which could be attributed to the radiation (tables IIIb to IXb). This was true even for subjects exposed to 518 rad, a dose which was lethal for all eight of the subjects. Similarly, no enzyme-level alteration could be attributed to pulsed-neutron irradiation in the two lower dose levels (359 rads and 218 rads). At higher dose levels, however, some interesting results were obtained.

At a dose of 518 rads, SGOT in survivors appeared to be consistently elevated over the controls after the first 6 hours. Surprisingly, nonsurvivors did not exhibit this marked increase in serum SGOT levels (table IIIa). Similar results were obtained with SGOT at 476 rads, (table VIIa), and with LDH at 518 rads (table Va) although, in the latter case, nonsurvivors also show some elevation. The behavior of SGPT was quite different, being markedly depressed below controls only in the case of nonsurvivors (table IVa). The malic dehydrogenase (MDH) showed no consistent pattern either of elevation or depression (table VIa).

It will be clear from the foregoing comments that the serum enzyme levels considered offer little promise as a quantitative biological indicator of absorbed radiation. As in the study of serum zinc levels, the spread in values obtained in any one group at one time, and the change in time of any one individual (including controls), are large. These day-to-day variations observed in sheep appear to be characteristic of man as well. The large variations in human serum zinc, reported above, have a counterpart in human serum enzyme levels. Normal human serum levels range from 8 to 40 standard units for SGOT, 5 to 30 units for SGPT and 200 to 650 units for LDH, (reference 30). Obviously a three- to six-fold variation in the normal population would obscure any effects resulting from radiation. These effects, if they are at all real and consistent, appear to be much smaller.

4. Individual Variations

The ubiquity of the phenomenon of individual variation has been discussed in detail (reference 31). Such variation is an inevitable consequence of the individual's heredity, history, and environment. Variation would thus be expected to pose even more problems in the analysis of human responses than in that of controlled experimental populations. Variations in serum enzyme levels are particularly well documented because of their importance in diagnostic tests.

Clinically, far greater alterations than those observed here are required for enzymatic diagnosis of pathological conditions. In myocardial infarction, for example, SGOT may be increased by a factor of 10 over the "normal" values, while LDH may increase by a factor of 5 to 15 (reference 30). Even in diagnostic techniques where such large increases are demonstrated, individual variation and effects unrelated to the condition being tested for may vitiate the results. It has been pointed out that

> "the SGOT activity cannot be used as a specific test for cardiac injury in patients suffering bodily trauma, inasmuch as over 50 percent of such injured patients may show elevated activity that is unrelated to demonstrable cardiac injury."

(quoted in reference 32). In addition it has been reported that a variety of strong stimuli will induce significant alterations in blood transaminase activity (reference 33). Both bodily trauma and strong psychological stimuli would certainly be expected in human populations exposed to large radiation doses. Further examples of serum enzyme alterations caused by sources other than radiation may be found in the reviews of Frajola et al; (references 34, 35) and Zimmerman and West (reference 36).

In view of the number of causes of serum enzyme alteration reported in the literature, it would be unjustified to claim that the results reported here clearly demonstrate a radiation-induced change. On the basis of the literature, we can certainly explain the day-to-day variation of the controls; the poor statistics obtained throughout the experiment can be attributed to handling of the animals, nonspecific injuries, feeding variations, etc. But at the same time, these explanations could also be advanced as the cause of the only slightly greater variation in the irradiated subjects.

One interesting point with respect to variation is that the controls for X-ray experiments are far more uniform than those for the neutron experiments. While the reason(s) for this difference cannot be stated with certainty, it is

probably a result of different handling of the two control groups. The X-irradiated sheep were merely moved about in the same general location as their holding pens, and were handled by personnel to whom they had become accustomed; but the neutron-irradiated sheep were transported by truck to a remote location, and were handled by personnel unfamiliar to them. Taking into account the reports cited above, as well as our own observations on the response of these animals to various stimuli, it is reasonable to suppose that the added stress to which the second group was exposed could account for the differences observed.

SECTION V

CONCLUSIONS

The experiments reported here indicate that individual variation and non-specific changes with time of the biological parameters measured render a "baseline" concept of diagnosis virtually useless so far as radiation damage is concerned. Because of the large range of normal (control) values, the appearance in a subject of an alteration in any of the parameters studied here cannot realiably be attributed to the effect of radiation. While the results regarded as a whole do suggest radiation-induced alterations in some serum enzyme levels, they are not quantitatively related to dose. In particular the radiation dose received by an individual cannot be determined from these parameters.

APPENDIX

THE SPECTROPHOTOMETRIC DETERMINATION OF ZINC IN BLOOD PLASMA OR SERUM

A. L. Dunn

T. J. Johnson

M. A. Hession

Omaha Veterans Administration Hospital

REAGENTS

Hydrochloric Acid (HCL)

Dilute reagent grade HCl to 10% by volume with demineralized water.

Trichloroacetic Acid (TCA)

Redistill reagent grade Trichloroacetic acid and make a 20% w/v solution with demineralized water.

Methyl Orange (indicator solution)

0.1% aqueous solution.

Ammonium Hydroxide (NH_4OH)

Redistill reagent grade ammonia into demineralized water. A 5N solution can be obtained in this manner. The working solution should be diluted to 1N.

Acetate Buffer

Mix equal volumes of 2N sodium acetate and 2N glacial acetic acid and adjust pH to 4.75. Remove the reacting heavy metals by shaking with 0.005 or 0.01% Dithizone (dephenyl thiocarbazone) in carbon tetrachloride. Filter to remove droplets of Dithizone.

Sodium Thiosulfate ($Na_2S_2O_3$)

Dissolve 25 g $Na_2S_2O_3 \cdot 5H_2O$ into 100 cc of demineralized water. Remove reacting heavy metals by extraction with Dithizone/CCl_4 . 0.005 or 0.01%.

Diphenylthiocarbazone (Dithizone)

Make a stock solution 0.01% w/v in FISHER SPECTRANALYZED CARBON TETRACHLORIDE. Stored in an amber bottle under refrigeration, the reagent remains stable over 6 weeks. The working solution, 0.001%, can be prepared from this daily or weekly as needed.

Standard Zinc Solution

0.100 mg/ml in 0.2N HCl. Prepared by dissolving 10 mg of 30-mesh reagent grade zinc metal in 5 ml of 4N HCl and diluting to 100 ml in a volumetric flask. Allow to stand several days before use. Standards for curves are made from this solution by the proper dilution.

APPARATUS

Beckman Model DU Spectrophotometer
Silica cells, 10 mm light path, covered
125 ml. capacity Squibb separatory funnels with teflon stopcocks
Glass-tipped 20 cc syringes

All glassware except the silica absorption cells is washed in a suitable laboratory detergent; rinsed with tap water, soaked at least 3 hours in 2N nitric acid, rinsed with demineralized water and oven-dried.

Water used throughout this procedure is obtained by running distilled water through Barnstead mixed bed resin. This is referred to as demineralized water.

PROCEDURE

1. Collect 12-20 cc of venous blood in a 20 cc heparinized syringe (this amount of blood yields sufficient plasma or serum for replicate determinations). Donor need not be fasting.
2. Transfer to a test tube containing 1-2 drops of heparin. Cover with parafilm and mix gently.
3. Centrifuge at 3000 rpm for about 15 minutes.
4. To 2 cc of nonhemolyzed plasma or serum in a centrifuge tube, add 2 cc of 10% HCl, mix well and permit to stand 10 minutes.
5. Next add 2 cc of 20% TCA, again mix well and permit to stand 10 minutes.
6. Cover the tubes with parafilm and centrifuge at 3000 rpm for 30 minutes.
7. Pipette a 3 cc aliquot of the clear supernatant into a separatory funnel, add one drop of methyl orange, and titrate to the color change (pH 3.5) with NH_4OH.

8. Bring total volume to 10 cc with demineralized water.
9. Add 5 cc of acetate buffer and 1 cc of 20% sodium thiosulfate.
10. DARKEN ROOM - so that there is no direct lighting. Darkroom lamps may be used.
11. Add 5 cc of 0.001% Dithizone (from aburet) to the contents of the separatory funnel and shake vigorously for 2 minutes.
12. Plug the funnel stem with glass wool, fill and cover the silica curvettes.
13. Read optical density at 520 microns. Avoid exposure to strong light.
14. Reagent blank - steps 4 - 13. Replace serum or plasma with demineralized water.
15. Standard curve - from standard stock solution make concentrations of zinc corresponding to 0.5, 1.0, 1.5, 2.0 and 2.5 µg/cc. Follow steps 4 - 13 using these standards in place of plasma or zinc.
16. DU set at zero optical density with reagent blank.

RESULTS

Reproducibility Study: Of a total group of 30 samples, determined gravimetrically to contain 4.0 µg of zinc, the following reproducibility data were obtained.

Mean value	4.00 µg
Standard deviation	0.05

Three readings above 4.05 (4.08, 4.09, 4.06)
Five readings below 3.95 (3.93, 3.86, 3.93, 3.93, 3.93)

Recovery: A large pool of plasma was split into two series: a control series and a series composed of controls to which 1.00 µg of zinc was added. Based on the results of the controls, the recovery was calculated in the following manner.

$$\% \text{ Recovery} = \frac{\text{Actual Recovery}}{\text{Calculated Recovery}} \times 100$$

$$\% \text{ Recovery} = 100.3\% \pm 2.1$$

REFERENCES

1. Wolff, H., "Der normale Zinkylbalt in Blut, Serum und Erythrocyten," Deut Arch Klin Med., 197:263 (1950).

2. Peterson, D. F., "Acute Radiation Death Resulting from an Accidental Nuclear Critical Excursion," J. Occ. Med., 3:155 (1961).

3. Peterson, D. F. and L. B. Hughes, "Serum Enzyme Content Following X-ray and Gamma-Neutron Irradiation," Rad. Res, 9:166 (1958).

4. Brent, R. L., M. M. McLaughlin and J. N. Stabile, "The Effect of Irradiation on the Serum Glutamic-Oxalacetic Transaminase Level," Rad. Res., 9:24 (1958).

5. Bean, W. B., R. W. Vilter and T. D. Spies, "The Effect of Roentgen Ray on the Blood Codehydrogenase, I, II": Ann. Int. Med., 13:783 (1939).

6. Deutsch, P., "Alier die Wirkung der Roentgenstrahlen die Katulase des Blutes," Strahlentherapie, 48:114 (1933).

7. Bernard, R. D., "A Note on Depression of Cholinesterase Level Following X-ray Therapy and Its Bearing in the Mechanisms of Radiation Sickness," Med. Rec., 161:16 (1948).

8. Peterson, D. F., C. C. Lushbaugh and P. Lee, "Serum Glutamic-Oxalacetic Transaminase Activity in Irradiated Animals," Fed. Proc., 16:327 (1957).

9. Brucer, M., The Acute Radiation Syndrome, ORINS 25, Oak Ridge Institute of Nuclear Studies (1959).

10. Hawrylewicz, E. J., Development of a Simple Laboratory Procedure to Detect Early Radiation Damage, WADD-TR-60-622, Wright Air Development Division, USAF, (1960).

11. Melch, I. J., and H. G. Albaum, "Serum Transaminase Activity in X-irradiated Rabbits," Proc. Soc. Exp. Biol. Med., 93:595 (1956).

12. Kessler, G., M. B. Hermel, and J. Gershom-Cohen. "Serum Glutamic-Oxalacetic Transaminase Activity after Whole-Body Irradiation," Proc. Soc. Exp. Biol. Med., 98:201 (1958).

13. Albaum, H. G., "Serum Enzymes Following Whole-Body Radiation in the Rat," Rad. Res., 12:186 (1960).

14. Vallee, B. L. and F. L. Hock, "Zinc, a Component of Yeast Alcohol Dehyrogenase," Proc. Nat. Acad. Sci., 41:327 (1955).

15. Vallee, B. L. and H. Neurath, "Carboxypeptidase, Zinc Metalloenzyme," J. Biol. Chem., 217:253 (1955).

16. Vallee, B. L. and F. L. Hock, "Zinc in Horse Liver Alcohol Dehydrogenase," J. Biol. Chem., 225:185 (1957).

17. Vallee, B. L., S. J. Adelstein and J. A. Olson, "Glutamic Dehydrogenase of Beef Liver, a Zinc Metalloenzyme," J. Am. Chem. Soc., 77:5196 (1955).

18. Kay, R. E., J. C. Early and C. Entenman, "Increased Urinary Excretion of Taurine and Urea by Rats after X-irradiation," Rad. Res., 6:98 (1957).

19. Stern, D. N. and E. M. Stim, "Sources of Excess Taurine Excreted in Rats Following Whole-Body Irradiation," Proc. Soc. Exp. Biol. Med., 101:125 (1959).

20. Cavalieri, R. R., M. Van Metre, F. W. Chambers and E. R. King, "Taurine Excretion in Humans Treated by Total-Body Radiation," J. Nuc. Med., 1:186 (1960).

21. Katz, E. J. and R. J. Hasterlick, "Amino Aciduria Following Total-Body Irradiation," J. Nat. Cancer Inst., 15:1085 (1955).

22. Hoberlein, A. B., E. D. Hubbard and R. Getty, "The Procurement and Handling of Swine Blood Samples on the Farm," J. Am. Vet. Med. Assoc., 119:357 (1951).

23. Jones, N. D., R. K. Jones & W. R. Godden, Average Ranges & Means of Blood Values in New Mexico Bred Sheep under Specified Environmental Conditions, AFWL-TR-65-109, Air Force Weapons Laboratory, USAF, (1965).

24. Mobley, T. S., et al., Median Lethal Dose, Studies on Sheep Following 250 KVP X-irradiation, AFWL-TR-65-200, (in preparation) Air Force Weapons Laboratory, USAF, (1966).

25. Mobley, T. S, R. E. Engle, W. R. Godden and V. T. Penikas, "Invivo and Air Dosimetry of Fission Sprectrum Neutrons," Biological Effects of Neutron and Proton Irradiations, 1:103 (1964).

26. Rice, E. W., "Spectrophotometric Determination of Serum Copper with Oxalylidihydrazide," J. Lab. Clin. Med., 55:325 (1960).

27. Wroblewski, F. and J. S. LaDue, "Latic Dehydrogenase Activity in Blood," Proc. Soc. Exp. Biol. Med., 90:210 (1955).

28. Ochoa, A., "Malic Dehydrogenase from Pig Heart," Methods in Enzymology. Vol 1, Editors: S. Calmichant, N. Kaplan, Academic Press, New York, 1955.

29. Reitman, S. and S. Frankel, "A Colorimetric Method for the Determination of Serum Glutamic-Oxalacetic and Glutamic-Pyruvic Transaminases," Amer. J. Clin. Path., 28:56 (1957)

30. Long, C., Biochemist's Handbook, van Nostrand, Princeton, 1961.

31. Williams, R. J., Biochemical Individuality, Academic Press, New York, 1959

32. Roberts, H. J., Difficult Diagnosis, Saunders, Philadelphia, 1958

33. Blyuger, A. F., M. L. Belen'kii and Ya. Ya. Shuster, "Mechanism of Increased Serum Enzyme Activity during Action of Strong Stimuli," Vopr. Medi. Khim., 10:12 (1964), trans. fr. Russian, Fed. Proc.-Trans. Sup., 24:T93 Part II, (1965)

34. Frajola, W. J., E. Meyer-Arendt and J. Waltz, "Serum Enzymes and Bio-Chemical Individuality," Fed. Proc., 19:46 (1960).

35. Frajola, W. J., E. Meyer-Arendt and J. G. Bontschs, "Biochemical Patterns of Disease," Proc. Amer. Soc. Cancer Res., 3:112 (1960).

36 Zimmerman, H. J. and M. West, "Serum Enzymes in Gastrointestinal Diseases," Med. Clin. No. Amer., 48:189 (1964).

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)*

Air Force Weapons Laboratory
Kirtland AFB, New Mexico

2a. REPORT SECURITY CLASSIFICATION

Unclassified

2b GROUP

3. REPORT TITLE

EFFECTS OF RADIATION ON SOME SERUM ENZYMES AND TRACE ELEMENTS IN LARGE ANIMALS

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

1 April 1962 to 7 June 1965

5. AUTHOR(S) *(Last name, first name, initial)*

Riggsby, W. S., 1 Lt, USAF; Jones, N. D., Capt, USAF; Godden, W. R., Maj, USAF

6. REPORT DATE	7a TOTAL NO. OF PAGES	7b. NO. OF REFS
January 1966	42	36

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. 7801	AFWL-TR-65-112
c. TASK 780104	9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
d.	

10. AVAILABILITY/LIMITATION NOTICES

Distribution of this document is unlimited.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	AFWL (WLRD) Kirtland AFB, NM

13. ABSTRACT

This report summarizes the results of determinations of serum zinc, copper, malic dehydrogenase (MDH), lactic dehydrogenase (LDH), glutamic-oxalacetic transaminase (SGOT), and glutamic-pyruvic transaminase (SGPT) in large mammals following various types and doses of radiation. The principal subjects were mature sheep although beagle dogs and "miniature" swine were also used. The radiation sources were a cobalt-60 teletherapy unit, a 250-kvp X-ray therapy unit, and a Godiva II pulsed fission-spectrum neutron reactor. Results indicate that the quantity and type of irradiation received by these animals cannot be determined from these parameters.

DD FORM 1 JAN 64 1473

Unclassified

Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Sheep - radiation effects on						
Serum-enzymes						
Trace elements						
Glutamic-oxalacetic transaminase (SGOT) (GOT)						
Glutamic-pyruvic transaminase (SGPT) (GPT)						
Malic dehydrogenase (MDH)						
Lactic dehydrogenase (LDH)						
Zinc						
Copper						
Metalloenzymes						
Cobalt-60						
X-ray						
Neutrons						

INSTRUCTIONS

1. ORIGINATING ACTIVITY: Enter the name and address of the contractor, subcontractor, grantee, Department of Defense activity or other organization *(corporate author)* issuing the report.

2a. REPORT SECURITY CLASSIFICATION: Enter the overall security classification of the report. Indicate whether "Restricted Data" is included. Marking is to be in accordance with appropriate security regulations.

2b. GROUP: Automatic downgrading is specified in DoD Directive 5200.10 and Armed Forces Industrial Manual. Enter the group number. Also, when applicable, show that optional markings have been used for Group 3 and Group 4 as authorized.

3. REPORT TITLE: Enter the complete report title in all capital letters. Titles in all cases should be unclassified. If a meaningful title cannot be selected without classification, show title classification in all capitals in parenthesis immediately following the title.

4. DESCRIPTIVE NOTES: If appropriate, enter the type of report, e.g., interim, progress, summary, annual, or final. Give the inclusive dates when a specific reporting period is covered.

5. AUTHOR(S): Enter the name(s) of author(s) as shown on or in the report. Enter last name, first name, middle initial. If military show rank and branch of service. The name of the principal author is an absolute minimum requirement.

6. REPORT DATE: Enter the date of the report as day, month, year; or month, year. If more than one date appears on the report, use date of publication.

7a. TOTAL NUMBER OF PAGES: The total page count should follow normal pagination procedures, i.e., enter the number of pages containing information.

7b. NUMBER OF REFERENCES: Enter the total number of references cited in the report.

8a. CONTRACT OR GRANT NUMBER: If appropriate, enter the applicable number of the contract or grant under which the report was written.

8b, 8c, & 8d. PROJECT NUMBER: Enter the appropriate military department identification, such as project number, subproject number, system numbers, task number, etc.

9a. ORIGINATOR'S REPORT NUMBER(S): Enter the official report number by which the document will be identified and controlled by the originating activity. This number must be unique to this report.

9b. OTHER REPORT NUMBER(S): If the report has been assigned any other report numbers *(either by the originator or by the sponsor)*, also enter this number(s).

10. AVAILABILITY/LIMITATION NOTICES: Enter any limitations on further dissemination of the report, other than those imposed by security classification, using standard statements such as:

(1) "Qualified requesters may obtain copies of this report from DDC."

(2) "Foreign announcement and dissemination of this report by DDC is not authorized."

(3) "U. S. Government agencies may obtain copies of this report directly from DDC. Other qualified DDC users shall request through _____."

(4) "U. S. military agencies may obtain copies of this report directly from DDC. Other qualified users shall request through _____."

(5) "All distribution of this report is controlled. Qualified DDC users shall request through _____."

If the report has been furnished to the Office of Technical Services, Department of Commerce, for sale to the public, indicate this fact and enter the price, if known.

11. SUPPLEMENTARY NOTES: Use for additional explanatory notes.

12. SPONSORING MILITARY ACTIVITY: Enter the name of the departmental project office or laboratory sponsoring *(paying for)* the research and development. Include address.

13. ABSTRACT: Enter an abstract giving a brief and factual summary of the document indicative of the report, even though it may also appear elsewhere in the body of the technical report. If additional space is required, a continuation sheet shall be attached.

It is highly desirable that the abstract of classified reports be unclassified. Each paragraph of the abstract shall end with an indication of the military security classification of the information in the paragraph, represented as *(TS)*, *(S)*, *(C)*, or *(U)*.

There is no limitation on the length of the abstract. However, the suggested length is from 150 to 225 words.

14. KEY WORDS: Key words are technically meaningful terms or short phrases that characterize a report and may be used as index entries for cataloging the report. Key words must be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location, may be used as key words but will be followed by an indication of technical context. The assignment of links, rules, and weights is optional.