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LATE EFFECTS OF SUBLETHAL LEVELS OF RADIATION ON GROWTH AND NUTRIENT DIGESTION IN BURROS

A Dissertation

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

by

Joseph John Knapka

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CHAPTER I

INTRODUCTION

Detailed studies concerning the physiological, biochemical, and genetic effects of radiation on numerous biological systems have been conducted. However, a large percentage of these studies have dealt with the acute effects that occur following exposure to a lethal dose of ionizing radiation. In general, the late effects of a sublethal dose of radiation on mammals comparable in size to man have not been studied extensively. In particular, possible alterations in the ability of young mammals to grow and develop after recovery from the acute effects of a sublethal dose of whole-body irradiation have received limited attention.

Knowledge concerning the late effects of radiation on large animals has practical significance since man and/or his food supply may be exposed to varying levels of radiation either by accident or design. Data concerning possible late effects of sublethal levels of radiation on growing mammals would be valuable from the medical standpoint in the event that children were exposed to various levels of radiation. These data could also aid in predicting the amount of human food that would be available from growing animals that survive.

Because the ultimate objective of most research involving the effects of radiation on biological systems is to collect data that can be extrapolated to man, the burro (<u>Equus Asinus</u>) is a particularly good subject for studies designed to determine the effects of whole-body radiation. Anatomically, all vital radiosensitive body systems and organs of both burro and man are in a similar geometric position in relation to a radiation source, even though the burro is somewhat larger in body mass. From a physiological standpoint, man and burro have monogastric digestive systems, relatively long life spans, and both are exposed to similar environmental variations. Economic advantages for using burros as subjects for studies with long-term end points are that they can utilize large quantities of inexpensive feedstuffs and that they do not require any elaborate facilities or housing.

The primary objective of the present work was to determine possible alterations in growth and in nutrient digestion of burros that have recovered from the acute effects of a sublethal dose of whole-body ionizing irradiation.

The objectives of the growth study were: (1) to evaluate the effects of whole-body irradiation on the subsequent performance of burro foals that were exposed to a sublethal dose of whole-body irradiation during their fourth month of life and (2) to test the response of irradiated and nonirradiated foals to three levels of dietary protein. Protein was selected as the varying nutrient because, of the major nutrients, protein supplies are the most limited throughout the world. In times of stress any shortage of a major nutrient would become more critical than during normal periods. Thus data obtained concerning the response of irradiated animals to various levels of dietary protein would be of greater value than data concerning more abundant nutrients.

The possibility exists that radiation-induced damage in the gastrointestinal system of the burro may be permanent in nature (Brown, Johnson, and Cross, 1965). Therefore, the present study included trials that were designed to detect possible alterations of nutrient digestion in burros that had survived a sublethal dose of whole-body irradiation for extended periods of time.

The secondary objective of these experiments was to make a contribution to the data in equine nutrition that is presently being accumulated. Since various levels of nutrients were used, results of these experiments, involving a relatively large number of animals as far as the equine species is concerned, may have wide practical application if these results are extrapolated to the horse. The practice of using smaller animals for nutritional pilot experiments and extrapolating the results to larger animals is quite common; examples include miniature pigs for swine and pigmy goats or sheep for larger ruminants.

CHAPTER II

REVIEW OF LITERATURE

A brief history of man's interest in radiation sickness has been presented by Kurtsin (1963). This Russian author states that isolated cases of radiation lesions were noted soon after Roentgen discovered X rays in 1895. In 1896 Becquerel and P. Curie found burns on their hands caused by radium rays. The first actual case of radiation sickness was described 6 years later. There were 7 such cases reported in 1907, 31 in 1908, and 54 in 1911.

Nine deaths were recorded between 1922 and 1924 among women painting watch dials with paints containing radioactive substance. Martland, Conlon, and Knef (1925) noted that the decedents had worked almost constantly at painting dials for from 3 to 7 years. These authors noted that the women could actually ingest up to 1.75 gm. of paint per day by continually pointing the camel's hair brushes with their lips. It was calculated that these women had swallowed 5 mg. of radioactive substance every 6 months. This paper also included the first report of a necropsy performed on the body of a person whose death was known to be associated with radiation sickness. Martland (1929) presented detailed descriptions of 18 fatalities that were believed to be caused by ionizing radiation.

The detonation of atomic bombs over Hiroshima and Nagasaki emphasizes to the present generation the importance of ionizing radiation on biological systems. Large numbers of people who were exposed to those atomic blasts have been examined by various medical experts and the irradiation syndrom has been characterized by marked impairment of digestive functions, nervous, cardiovascular and sex disorders, hemorrhagic tendencies, dystrophy, leukopenia, anemia, leukemia, and adynamia (Warren, 1946; LeRoy, 1947 and 1950; Liebow, Warren, and DeCoursey, 1949; Cronkite, 1951; Kusano, 1953).

Acute Effects of Irradiation on the Gastrointestinal Tract

Clinical symptoms. It is well documented that the gastrointestinal tract is one of the most radiosensitive of the biological systems. The clinical symptoms of radiation injury of the digestive system are very pronounced and for this reason radiation damage to this system has been studied extensively.

Digestion disorders of humans due to radiation injury include marked anorexia, nausea, vomiting, meteorism, disturbed secretory and motor activity of the tract, stomach pains, acute weight loss, and several local and general inflamations in the alimentary canal (Warren and Friedeman, 1942; LeRoy, 1947). Kurtsin (1963) cites cases where some of these symptoms are exhibited by individuals who receive therapeutic localized irradiation as well as those exposed to whole-body irradiation. This author also states that symptoms occur soon after exposure; one man suffered nausea and vomiting within 20 seconds after a 300 roentgen (R) dose of gamma whole-body irradiation.

Conard (1956) postulated that symptoms related to the gastrointestinal tract are reflected _via_ a complex system of nervous control through the autonomic sympathetics and parasympathetics, and that the gut has an enteric nervous system of its own. As a result, gastrointestinal symptoms may stem from diseases of the gastrointestinal tract itself, from diseases of other parts of the body _via_ complex nerve pathways, from impulses arising in the central nervous system, or from a combination of these sources. He suggested that gastrointestinal symptoms that occur soon after whole-body irradiation or from localized therapeutic irradiation may be similar to the phenomenon of "psychic" vomiting which may be produced by the sight of blood, extreme fear, or a shocking experience rather than by direct damage to the gut.

Even though gastrointestinal symptoms first exhibited following radiation may be secondary, there is a large volume of literature regarding direct radiation injury of the gastrointestinal system. Bond _et al._ (1950) credits the French workers Regaud, Nogier, and Lacassagne (1912) for first experimentally demonstrating the exceptional radiosensitivity of the abdomen. Warren and Whipple (1922) presented an extended discussion of the radiosensitivity of the intestinal tract of dogs. In their necropsy report Martland, Conlon, and Knef (1925) reported intestinal lesions in the body of a woman they believed to have died from radiation toxicity. Since these early observations of radiation damage to the gastrointestinal system, numerous workers have noted gastrointestinal symptoms or damage in most species of animals that were exposed to radiation.

Conard (1956) has characterized the gastrointestinal tract symptoms for a sublethal dose of irradiation as anorexia, nausea, and vomiting with a slight loss in body weight. For a lethal dose these symptoms are more pronounced. If the dose is in the supralethal range, additional symptoms consist of marked diarrhea, bloody diarrhea, dehydration, and ulceration of the tract. Symptoms quite similar to those outlined by Conard (1956) generally have been reported for most species. Species of animals that have exhibited gastrointestinal symptoms as a result of exposure to various levels of radiation include mice (Quastler _et al._, 1951; Chapman, 1955), rats (Bond _et al._, 1950; Smith and Tyree, 1954), chicks (Vogel and Steaner, 1955), dogs (Brecher and Cronkite, 1951; Handford, 1960), primates (Allen _et al._, 1960), cattle (Brown _et al._, 1961; Brown, 1962), burros (Trum _et al._, 1953; Trum _et al._, 1959a), and swine (Tullis, Lamson, and Madden, 1954; Chambers _et al._, 1964). Smith, Ackerman, and Smith (1952) and Trum _et al._ (1959b) irradiated guinea pigs and swine, respectively, and they did not report typical clinical symptoms of intestinal injury. Quastler (1956) described the symptoms of deaths that are attributed to irradiation injury of the gastrointestinal system.

Functional effects. Alterations in intestinal function associated with the acute radiation syndrome have been studied by various workers. Results of these studies generally indicate a decrease in sugar absorption (Detrick _et al._, 1955; Farrar _et al._, 1956; Perris, Jervis, and Smyth, 1966). Bennett _et al._ (1951) and Mehran and Blais (1966) reported a slight inhibition of protein absorption as a result of irradiation. However, Bennett et al. (1950) have attributed this small difference to the prolonged emptying time of the stomach and altered intestinal motility that is associated with the irradiation syndrome. Mead, Decker, and Bennett (1951) and Sullivan (1961) presented data that indicated fat absorption is not altered by radiation. Radiation reduced the intestinal uptake of sodium in the rat (Goodner et al., 1955; Curran, Webster, and Hovsepian, 1960) but increased the uptake of ^{45}Ca and ^{85}Sr in this species (Lengemann, 1960; Lengemann and Comar, 1961). Lowrey and Bell (1964) reported that irradiated pigs retained more ^{89}Sr than the non-irradiated controls, but the retention of ^{45}Ca was the same for both groups of pigs.

Histological alterations. The histopathology of irradiation on numerous biological systems has been reviewed by Bloom (1948). His section regarding the gastrointestinal tract includes the early work reported in this field which is in general agreement with results published more recently (Baker and Mitchell, 1963; Perris, Jervis, and Smyth, 1966; Wiernik, Creamer, and Shorter, 1966). On the first day following a sublethal dose of whole-body irradiation the contents of the villi begin to shrink away from the epithelial layer, the cells of this layer are slightly swollen, and there appear to be no abnormalities in the crypts. The shrinkage continues for 2 days after irradiation and fluid-filled spaces appear in the submucosa. Epithelial cells are swollen and in the crypts the nuclei appear large. The villi have shortened somewhat, but in spite of the degenerative process the epithelial layer is more or less intact. There are fewer cells in the crypts and around the villi. After 3 days the villi are still contracted compared with sections from the control animals. Proliferation is resumed in the crypts and there are few, if any, abnormal divisions. Epithelial cells are more numerous, and the epithelium is intact. Cells are still somewhat enlarged and pale, but the fluid-filled spaces in the submucosa have now vanished. After 4 days the villi have resumed their normal shape and are densely covered with epithelial cells packed very closely together; the crypts are normal. After 6 days no differences can be detected between the appearance of the intestine from an irradiated and a nonirradiated animal. In some experiments the intestine was examined 18 and 21 days after radiation and showed no differences from the normal intestine. It appears that the greatest histological changes in the gastrointestinal tract occur 2 or 3 days after irradiation.

Factors Affecting Radiation Damage

A considerable amount of effort has been expended in the search for an agent that would provide radiation protection for biological systems. A multitude of natural and synthetic chemicals have been tested for this purpose with varying degrees of success. However, since the major objectives of the present study were nutritional in nature, this review will be limited to the effects of age and nutritional status of the animal on the degree of irradiation injury.

<u>Age</u>. Reports of studies involving whole-body irradiation usually are not concerned with the age of the animals that are radiated, yet radiotherapists have recognized for many years the effect of age on the sensivity of man to ionizing radiation. Lindop and Rotblat (1962) reviewed literature concerning the susceptibility of differently aged rodents to whole-body ionizing radiation. The reports reviewed usually described effects on the younger ages and showed that weanling rats and mice were most sensitive to radiation, whereas neonatal animals were less sensitive. Although the data for older ages were scant, it does suggest that the $LD_{50(30)}$ would vary with age throughout life in proportion to the remaining life expectation. That is, there was a rapid increase in sensitivity at old age.

Sikov and Noonan (1964) reported an $LD_{50(30)}$ of 360 R and 420 R for rats exposed to whole-body X irradiation when they were 21 and 32 days old, respectively. Animals exposed when 60 or 100 days old were not found to differ in sensitivity, the $LD_{50(30)}$ being 758 R for both ages combined. They stated that the change in sensitivity from weaning to maturity seemed to be greater in the rat than in the mouse. Although actual ages were not reported, Chambers <u>et al</u>. (1964) irradiated 75 and 150 lb. swine and found no statistically significant differences in the sensitivity of the two groups. However, if only the air dose and weight were considered rather than tissue dose and its distribution, then there was a statistically significant difference in the response of the two weight classes, with the lighter group being the most radiosensitive.

<u>Nutritional</u> <u>status</u>. Jennings (1949) reported the results of the first extensive study regarding the effect of radiation on animals that were fed various levels of a major nutrient. He found that if rats weighing 200 gm. were fed a low protein diet until they lost 25 per cent of their weight and then irradiated, the $LD_{50(30)}$ was 520 R as compared to 700 R for rats fed a normal diet. Later Smith, Ackerman, and Alderman (1952) reported that rats maintained before and after radiation on diets containing only 6 per cent casein showed a slightly higher incidence of death after irradiation with 550 R than did rats fed 18 and 35 per cent casein diets. Mortality was not altered if the casein content of the diet was decreased after irradiation, but if it was increased to 60 per cent the observed mortality was reduced. Recently, Hopper, Yatvin, and Wannemacher (1966) also concluded that a low protein diet fed to adult rats after a sublethal dose of irradiation did not effect the repair process. However, the protein reserves of the rats had not been depleted in this particular study.

Cheng <u>et</u> <u>al</u>. (1952) reported no differences in the susceptibility of rats to X-irradiation injury as related to the fat content of the diet when rats fed the experimental diets for 3 or 4 weeks were exposed to 650 or 850 R in a single dose. Yet rats receiving fat in their diets for 12 weeks were found to be more radiation resistant than rats fed a fat-free diet when 2,400 R were administered in 300 R doses over a 10- to 14-week period. Ershoff (1960) also reported that the average survival time of mice exposed to multiple sublethal doses of whole-body X irradiation was significantly less on a fat-free diet than on a similar diet supplemented with 10 per cent cottonseed oil. Carroll and Brauer (1961a) presented data which indicated that the radiation resistance of hypocalorically reared rats is significantly reduced as compared to _ad libitum_ fed controls, and that the decreased radiation resistance could not be attributed to the size of the rats or to the stretching out of the life span of the hypocalorically reared rats.

It has been speculated that the loss of weight and perhaps the death of some animals following exposure to radiation might be attributed to anorexia. Smith, Chapman, and Alderman (1952) radiated mice that were made fat by goldthioglucose injection and mice with hereditary adiposity. They observed the same incidence of deaths in these mice following whole-body irradiation as was observed in control mice of normal size. The results of this study suggested that starvation is not responsible for death in mice following radiation. Forced feeding of animals after radiation in order to sustain nutrition has been detrimental rather than helpful (Smith, Ackerman, and Smith, 1952; Smith and Tyree, 1954; Anonymous, 1956; Akin, Coniglio, and Hudson, 1957).

Ziporin _et al._ (1959) demonstrated that postirradiation anorexia is responsible for the characteristic loss in body weight that occurs during this period. Weanling male rats were paired according to weight and one rat of each pair was exposed to 725 R of X irradiation. The irradiated rats were fed _ad libitum_ and the food intake of each non-irradiated mate was restricted to this level. Feed consumption of irradiated rats had decreased to 20-50 per cent of the preirradiation levels by the third day after irradiation, and they had lost 2-6 per cent of their body weight. The same percentage of weight loss was reported for the nonirradiated controls. Under this feeding regime no significant difference in body weight was observed between the irradiated and nonirradiated rats at 7, 15, 20, or 28 days postirradiation.

Late Effects of Whole-body Irradiation

Effects on the aging process. Most controlled experiments involving the late effects of radiation have been designed to obtain data related to the life-shortening action of radiation. The relationship between the aging process and sublethal levels of irradiation has been reviewed in detail by Upton (1957 and 1960). In general, it has been shown that the life span is shortened as a result of exposure to ionizing radiation in proportion to the amount of radiation absorbed. The mode of action of radiation in accelerating the aging process is not understood.

Effects on the gastrointestinal tract. Relatively few experiments have been designed to determine the possible late effects of sublethal levels of whole-body irradiation on the ability of animals to utilize various foodstuffs. The most publicized evidence that suggests the gastrointestinal tract is permanently altered because of radiation injury can be found in the medical history of the victims of the Hiroshima and Nagasaki atomic bomb blasts. Ide et al. (1965) reported that between the years 1957-1961 the most frequently reported primary neoplasms in Hiroshima and Nagasaki populations in both sexes were of the stomach. Among a life-span study sample of these populations, 37 per cent of all malignant neoplasms were of the stomach.

Brown, Johnson, and Cross (1965) presented data which suggests that, at least in the burro, the damage of the gastrointestinal tract associated with acute effects of irradiation might be permanent in nature. They reported that death of burros that had survived various levels of irradiation for several years is often preceded by a loss in body weight and less frequently emaciation, although appetite is not inhibited. At necropsy necrotic lesions were found in the gastrointestinal tracts of these animals. These conditions cannot be associated with any specific pathological or clinical finding. A high incidence of gastroenteritis and loss of weight prior to death in both burros and pigs that had survived the acute effects of irradiation has been reported by Brown and Johnson (1966).

Effects on growth. Carroll and Brauer (1961b) designed an experiment that allowed the determination of the late effects of irradiation on the growth of rats without confounding the results with acute effects. Weanling rats were placed on a hypocaloric diet that was adjusted so each rat was allowed to reach a maximum weight of 125 gm. at no less than 3 months of age. At 3 months of age half the rats were irradiated and both irradiated and nonirradiated rats were maintained on the hypocaloric diet for as long as 6 months postirradiation and then returned to an adequate caloric intake. After a 25-week period of "refeeding," the final body weight of the nonirradiated rats was about 15 per cent greater than the irradiated group. The food intake was identical for both groups during the first 7 weeks of "refeeding" at which time the feed intake of the irradiated rats leveled off at 12-13 gm. per rat per day while the food intake of the nonirradiated group continued to increase for several weeks and then leveled off at about 15-16 gm. per rat per day. When the daily food intake was identical, the body weight increases of nonirradiated rats was significantly greater than that shown by the irradiated individuals, indicating higher nutrient utilization in the nonirradiated group. Essentially, the same results were obtained when the rats were exposed to single doses of X rays ranging from 400-700 rad. or to repetitive exposures of 200 rad. in four doses spaced 1 week apart. Shielding of the gastrointestinal tract during irradiation had no recognizable effect in preventing low gains of body weight for the irradiated rats, even though food intake remained the same for both groups of rats throughout the "refeeding" period. These authors concluded that the study involving shielding of the gastrointestinal tract during irradiation provided evidence which suggests that this is not the target area concerned with the reduced growth of the irradiated rats.

Goldwater and Entenman (1959) irradiated rats that were 5 weeks old (average weight 99 gm.) with three weekly fractions of 300 R each. After exposure to the first fraction of radiation, both irradiated and control rats were fed a fat-free ration, one containing corn oil or one containing butterfat. Through the first 10 weeks postirradiation, irradiated and nonirradiated rats gained the same amount of weight. Over a 26-week period irradiated rats gained 20 per cent less weight than the nonirradiated controls, even though the amount of feed consumed by the two groups did not differ. A similar effect was noted for each of the three rations. Both irradiated and nonirradiated rats fed the ration containing butterfat gained the most weight, while those fed the fat deficient ration gained the least weight. These authors concluded that feed consumption was not the prime factor responsible for the observed differences in growth between the irradiated and nonirradiated rats, and they suggested that some metabolic factors might be involved.

The observation that alterations in intermediary metabolism might occur as a result of irradiation is supported by the work of Lane *et al.* (1955) who reported a sharp decrease of expired CO_2 in burros receiving 200 R per day of gamma radiation. A similar decrease in CO_2 production was reported in rats exposed to a lethal dose of irradiation by Rust *et al.* (1963). However, the duration of the decreased expiration of CO_2 was not discussed by either group of authors.

Obviously, a concentrated effort has not been made to establish the latent effects of whole-body irradiation on nutrient digestion or utilization. The few reports that do appear concerning this subject suggest that several systems may be affected. The pathological reports from both humans (Ide *et al.*, 1965) and animals (Brown and Johnson, 1966) imply that there are alterations in the gastrointestinal tract *per se*. Work with rats (Carroll and Brauer, 1961b) indicates that appetite might be inhibited. Another study with rats (Goldwater and Entenman, 1959) suggests permanent injury to some metabolic system other than the gastrointestinal tract. Because of the possibility that several systems might be affected in animals that survive the acute effects of irradiation, it seems appropriate that the initial study in this area with large animals should be designed to determine the practical significance of possible alterations that occur.

CHAPTER III

EXPERIMENTAL PROCEDURE

The general objectives of the present work were to determine the late effects of irradiation on nutrient digestion and utilization. The growth study was designed to determine the effects of a sublethal dose of ionizing irradiation on the subsequent growth and development of burro foals fed three levels of protein. Criteria used to evaluate the treatments included body weight gains, feed consumption, feed efficiency, increase in height, and increase in heart girth, as well as the general condition of the foals. The first replication of this experiment included 18 irradiated and 18 nonirradiated burro foals that were fed rations containing 9, 14, and 18 per cent crude protein, while the second replication included 14 irradiated and 13 nonirradiated foals that were fed rations containing 9 and 18 per cent crude protein.

The digestion studies included a trial which was designed to determine the effect of age on nutrient digestion as well as three trials that were designed to test the late effects of irradiation on nutrient digestion. A total of 21 nonirradiated and 11 irradiated mature burros were used in the four digestion trials.

I. GROWTH STUDIES

Experimental Animals

Burro foals born to dams in the band maintained by the University of Tennessee-Atomic Energy Commission Agricultural Research Laboratory at Oak Ridge, Tennessee, were used in this experiment. The 36 foals used in the first replication were born in May and June of 1963, and the 27 used in the second replication were born in March and April of 1964. The mean birth weight of the foals in both replications was 21.3 kg. Dams and foals were maintained on a grass pasture and were supplemented with 0.45 kg. of concentrate per adult burro. No attempt was made to induce the foals to consume any feed other than the dams' milk and grass until weaning at 7 months of age.

Irradiation

While the foals were in their fourth month of life, they were given a complete physical examination by a laboratory veterinarian, and allotted into two groups according to weight, sex, and irradiation history of their dams. One group was then randomly selected to be exposed to a sublethal dose of whole-body gamma irradiation while the second group was designated as nonirradiated controls. In accordance with this design, the foals born in May and June of 1963 were irradiated on September 23 and October 23, 1963, respectively. Likewise, irradiation dates in 1964 were July 27 and August 31 for foals born in March and April, respectively.

The foals were subjected to a single exposure of 250 R (dose in air) whole-body ^{60}Co gamma radiation at the multicurie irradiation site described by Wilding, Rust, and Simons (1952). The radiation flux was approximately 30 R per hour as determined by the ^{60}Co decay curve.

It was estimated that this 250 R dose would induce alterations in the gastrointestinal system without causing a high incidence of mortality.

Factors considered in determining the 250 R dose included the $LD_{50(30)}$ dose of 784 R for the mature burro that was reported by Rust et al. (1954c), and the work of Lindop and Rotblat (1962) which indicated that the juvenile of a species is considerably more radiosensitive than the adult.

Postirradiation Procedure

First 30 days postirradiation. During this period rectal temperatures of the irradiated foals were recorded daily, blood samples were collected twice a week from the irradiated and weekly from the nonirradiated foals, and both irradiated and nonirradiated foals were weighed on a weekly schedule. Blood samples were analyzed by experienced technicians in the hematology section of the laboratory. Antibiotics were administered to the irradiated foals when rectal temperatures were above normal.

Days 31 through 90 postirradiation. Blood samples were collected and weights were recorded on all irradiated and nonirradiated foals on a biweekly schedule throughout this period. Counts were made of all blood cell types and when the cell populations of the irradiated foals equaled that of the nonirradiated foals, the foals were considered to have recovered from the acute effects of the radiation. The irradiated foals recovered from the acute effects of radiation, as determined by blood cell populations and body weights, when the average age of the foals was 7 months (90 days postirradiation). All foals were then weaned and the protein feeding experiment was initiated.

Procedure at Weaning and Allotting Foals to Treatments

All foals were declared in good physical condition by a laboratory veterinarian who examined each foal for any physical or anatomical defects, not associated with irradiation damage, that could have influenced the future performance of the foal. Analysis of blood, urine, and fecal samples indicated that all foals were free of organic disorders and internal parasites. Each foal was weighed on two consecutive days and the average of these two weights was used as the starting weight for the protein feeding experiment. The size of each foal was determined by measuring the heart girth and the height of the animal at the point of the shoulders. Each foal was hip branded to insure positive identification.

The 36 foals that were in the first replication were allotted into three groups according to irradiation treatment, weight, sex, size, and radiation history of their dams. Each group of foals was then randomly selected to be fed a ration that contained either 9, 14, or 18 per cent crude protein. The 27 foals that were available for the second replication were allotted into two groups according to the same criteria and then randomly assigned to be fed the rations containing either 9 or 18 per cent crude protein. The distribution of irradiated and nonirradiated male and female foals for both replications are presented in Table I.

Composition of Rations

The ration containing 14 per cent crude protein was based on feeding standards for the growing horse recommended by Morrison (1956) and was included as the control ration that supplied an adequate amount

TABLE I

EXPERIMENTAL DESIGN OF THE PROTEIN FEEDING EXPERIMENT INVOLVING IRRADIATED AND NONIRRADIATED BURRO FOALS

	Ration					
	9% crude protein		14% crude protein		18% crude protein	
Sex	Irradiated	Non-irradiated	Irradiated	Non-irradiated	Irradiated	Non-irradiated
			First replication			
Male	3	3	5	2	3	3
Female	3	3	1	4	3	3
			Second replication			
Male	3	3	-	-	3	3
Female	4	3	-	-	4	4

of protein. Because it was imperative for the three rations to be isocaloric, the lowest level of crude protein that could be formulated with the natural feedstuffs used in this experiment was 9 per cent.

The criteria for selecting ingredients used in the rations for this experiment included availability, consistency of nutrient composition, and physical characteristics. The rations consisted of soybean oil meal as a source of protein and cottonseed hulls as a source of crude fiber. Corn and cob meal was decreased as protein level increased. Wheat bran, alfalfa meal, molasses, salt, and mineral levels were constant among the three rations. The ingredients and the proximate analysis of each ration are shown in Tables II and III, respectively. Feed was ground and mixed at 2-week intervals during the 364-day duration of the experiment. The corn and cobs were ground to pass through the medium screen of a hammer mill before being mixed with the other ingredients. It was observed early during the feeding trial that the foals were able to sort out the cottonseed hulls from the more concentrated fraction of the rations; as a result the cottonseed hulls were also ground to insure a uniform consumption of the rations. Initially, the dust from the ground hulls was responsible for respiratory disorders in several of the foals. This problem was solved by limiting the amount of feed each foal was offered to approximately the amount that would be consumed in 1 hour.

Feeding Facilities and Management Procedure

The foals were weaned and transferred to a barn that was similar in structure to a loafing shed. The interior of the barn was divided

TABLE II

RATIONS CONTAINING VARYING LEVELS OF PROTEIN FED TO IRRADIATED AND NONIRRADIATED BURRO FOALS

Ingredients	Per cent protein in ration		
	9	14	18
	%	%	%
Corn and cob meal	51.50	37.75	25.25
Cottonseed hulls	21.25	21.25	22.50
Soybean oil meal	1.25	15.00	26.25
Wheat bran	15.00	15.00	15.00
Alfalfa meal (17%)	5.00	5.00	5.00
Blackstrap molasses	5.00	5.00	5.00
Dicalcium phosphate	0.50	0.50	0.50
Trace mineralized salt	0.50	0.50	0.50
Total	100.00	100.00	100.00

TABLE III

PROXIMATE ANALYSIS OF RATIONS CONTAINING VARYING LEVELS OF PROTEIN FED TO IRRADIATED AND NONIRRADIATED BURRO FOALS

Fraction	Per cent protein in ration		
	9	14	18
	%	%	%
Ash	3.85	4.52	5.06
Crude fiber	14.56	14.45	15.68
Ether extract	2.43	2.06	2.04
Nitrogen-free extract	70.07	65.05	59.01
Protein	9.09	13.92	18.21
Total	100.00	100.00	100.00

into two pens, and each pen was equipped with stalls that facilitated the individual feeding of each foal. A drylot containing an automatic water fountain was adjacent to each pen.

The foals were divided between the two pens according to sex; thus each pen housed both irradiated and nonirradiated individuals that were fed each of the three protein levels. Feeding stalls were assigned within the two pens so that the foals fed the rations containing the same protein level were adjacent to each other, but irradiated and nonirradiated foals within a protein subgroup were assigned to stalls at random. In a matter of days each foal was trained to enter the assigned stall and it was a simple chore to fasten a chain at the rear of the stall which held the foal in confinement until the chain was removed.

Throughout the 364-day feeding trial the foals were fed twice daily, at 7:00 a.m. and 3:00 p.m. The foals were confined to their assigned stalls and fed the appropriate experimental ration. One and one-half hours after each feeding, the foals were released from the stalls and given free access to the drylot and the area of the barn not occupied by the stalls until the next feeding period. Any feed that was not consumed was weighed and discarded after each feeding period, and the amount of feed each burro consumed was recorded.

In the absence of previous data regarding the amount of feed the growing burro would consume, the foals in this experiment were fed <u>ad libitum</u> rather than on a paired basis. Because feed consumption and growth records were maintained on each foal individually, any difference in feed efficiency due to irradiation could be determined. Therefore, the major advantage of feeding *ad libitum* was that each foal had an equal and independent opportunity to reach its production potential regardless of the irradiation treatment. This system of feeding also allowed the determination of the effect of irradiation on appetite and supplied data concerning the amount of feed required for the growing burro foal. Since a considerable amount of sorting occurred when an excess of feed was offered to the foals, a system that simulated *ad libitum* feeding was used. The amount of feed offered to a particular burro was 114 gm. more than was consumed at the feeding 24 hours earlier.

Data Collected During Feeding Trial

In addition to records concerning the amount of feed each foal consumed, foal weights were recorded on a 28-day schedule for the 364-day duration of the feeding trial. Since change in body weight is not always indicative of skeletal growth, height and heart girth measurements of each foal were recorded every 56 days. Blood samples were collected for hematological analysis each time the foals were weighed.

Maintenance of the Foals During the Period Following Protein Treatment

In order to obtain at least limited data regarding possible differences in the ability of irradiated and nonirradiated foals to recover from the nutritional stress, growth records were maintained for 156 days after the conclusion of the protein treatments.

The same facilities that were used during the protein trial were also used during this period. Basically, the feeding and management routine used during the protein trial was continued. The only alterations were that all foals were fed the same concentrate ration and a good quality alfalfa hay was fed _ad libitum_.

The concentrate ration consisted of the following ingredients: 54.8 per cent crimped oats, 20 per cent wheat bran, 10 per cent alfalfa meal, 5 per cent blackstrap molasses, 5 per cent soybean oil meal, 5 per cent ground limestone, and 0.2 per cent mineral mix. The proximate analysis of this ration as well as that of the alfalfa hay fed during this period are presented in Table IV.

Throughout this period the foals were weighed on a 28-day schedule and body measurements were recorded bimonthly. At the conclusion of this period the burros were returned to pasture with the laboratory band.

General Health Considerations

Throughout the duration of this study the foals were under daily observation by an experienced animal technician. Any signs of clinical abnormalities were diagnosed by the laboratory veterinarian who administered appropriate treatment. Clinical charts were maintained for each foal which included records of alterations in body temperature, clinical diagnosis, and administration of medication, as well as results of semiannual physical examinations which are routinely conducted on animals involved with irradiation studies.

The semiannual physical examination included a fecal analysis for parasite ova. When high ova counts were noted, the foals were treated with an oral dose of phenothiazine and hexanthelin to control internal parasites.

TABLE IV

PROXIMATE ANALYSIS OF FEEDSTUFFS FED TO FOALS DURING THE PERIOD FOLLOWING PROTEIN TREATMENT[a]

Fraction	Concentrate	Alfalfa hay
Ash	9.73	6.41
Crude fiber	11.29	31.09
Ether extract	3.33	2.12
Nitrogen-free extract	58.82	45.50
Crude protein	16.83	14.88
Total	100.00	100.00

[a]Air-dry basis.

If an animal's illness was serious enough that the well-being of other foals was jeopardized, the infected individual was removed to the laboratory animal hospital and confined until recovery. While in the hospital, the foal was fed the proper experimental ration and feed consumption was recorded. When a foal died, the laboratory pathologist performed a complete necropsy.

Performance data accumulated from foals that died were included in the analysis only for periods that were completed at the time of death.

Statistical Analysis

The basic experimental design of this study was a 2 x 2 x 3 factorial which was partially replicated. The method of least squares for analysis of data with disproportionate subclass numbers (Harvey, 1960) was used to evaluate treatment differences in performance traits. The model considered appropriate for this analysis was:

$$Y_{ijklm} = \mu + S_i + I_j + P_k + T_l + X_{ij} + Z_{jk} + e_{ijklm}$$

where

Y_{ijklm} = the observed value of the m^{th} foal of the i^{th} sex of the j^{th} irradiation level fed the k^{th} protein level in the l^{th} year.

μ = the theoretical population mean.

S_i = the effect of the i^{th} sex.

I_j = the effect of the j^{th} irradiation level.

P_k = the effect of the k^{th} protein level.

T_l = the effect of the l^{th} year.

X_{ij} = the interaction between the i^{th} sex and the j^{th} irradiation level.

Z_{jk} = the interaction between the j^{th} irradiation level and the k^{th} protein level.

e_{ijklm} = random error.

Feed consumption, feed efficiency, weight, and increase in height and heart girth were considered as the traits most indicative of treatment differences while the foals were fed the rations containing various levels of protein. The traits evaluated while the foals were fed the common ration during the postprotein period were gain in weight during the first 76 days, total gain for the period, and increase in both height and heart girth during the entire period.

Power of the test was calculated for those traits which did not exhibit significant treatment differences (Dixon and Massey, 1957).

II. DIGESTION TRIALS

The primary objective of the digestion trials was to determine possible alterations in nutrient digestion of burros that had survived sublethal doses of whole-body irradiation for extended periods of time. Since irradiation damage may accelerate the aging process (Upton, 1957 and 1960), it was essential to first establish the effect of age on nutrient digestion in nonirradiated burros to facilitate the evaluation of data obtained from irradiated and nonirradiated burros of the same chronological age.

General Procedures for Conducting Digestion Trials

Burros that were to be used in a digestion trial were confined to a shed-type barn which had an adjacent concrete exercise lot. During a 3-week period the high roughage ration that was normally fed was gradually replaced by the experimental ration that was relatively low in roughage. During the last 6 days of this period, the amount of the experimental ration that the burros would consume within 1 hour was determined. When the digestion trial was initiated, this established voluntary ration intake of each burro was slightly reduced to insure a complete and uniform feed consumption.

The digestion trial consisted of a 7-day preliminary period followed by a 10-day collection period. At the beginning of the preliminary period, the burros were placed in metabolism stalls that were similar in structure to those described by Hobbs, Hansard, and Barrick (1950). Throughout the trials burros were fed one-half their daily feed allotment at 7:30 a.m. and at 3:30 p.m. Water was offered just prior to and 1 hour after each feeding.

Fecal material was collected at 10:00 a.m. each day. It was weighed, mixed, and 5 per cent aliquots were placed in glass jars and frozen to prevent decomposition. At the end of the collection period the 10 daily aliquots from each burro were pooled and subsampled.

Trial I

This total-collection trial was designed to determine the effect of the burros' age on nutrient digestion. The five 2-year-old and the five 5-year-old nonirradiated male burros that were used averaged 184 and 204 kg. in body weight, respectively. During the digestion trial, each burro was fed 3.63 kg. per day of a ration consisting of 54.8 per cent crimped oats, 20 per cent wheat bran, 10 per cent alfalfa meal, 5 per cent blackstrap molasses, 5 per cent soybean oil meal, 5 per cent ground limestone, and 0.2 per cent mineral mix. The proximate analysis of this ration is presented in Table IV, page 29.

Trial II

This total-collection trial involved ten 12-year-old male burros. Five were nonirradiated and five had survived a single 500 R dose of whole-body gamma irradiation from a ^{95}Zr-^{95}Nb source for 7 years. The average weights of these animals at the beginning of the metabolism trial were 198 and 202 kg. for the irradiated and nonirradiated burros, respectively. These burros were also fed 3.63 kg. per day of the ration that was fed in Trial I.

Trial III

In this trial nutrient digestion was determined by using polyethylene as an external indicator. Generally, the same procedure used in the previous two trials was followed for this trial. However, instead of using metabolism stalls, the burros were placed in the barn equipped with individual feeding stalls. The burros were confined to these stalls for 1 hour following the 7:30 a.m. and 3:30 p.m. feedings. The remainder of the time they had free access to water in a drylot adjacent to the barn. Feed and feces polyethylene concentration was considered to have reached equilibrium during the 7-day preliminary period. Rectal fecal "grab" samples were collected from each burro at 8:00 a.m. and 4:00 p.m. during the 10-day collection period.

The burros used in this trial were four 3-year-old male burros that had been exposed to a single 250 R whole-body dose of gamma irradiation from a ^{60}Co source at 4 months of age and four nonirradiated male burros born the same year. When the trial was initiated, the average body weight of both irradiated and nonirradiated burros was 164 kg.

The amounts of feed fed daily ranged from 3.07 to 3.64 kg. per burro and the ration consisted of 34.2 per cent crimped oats, 31.2 per cent cottonseed hulls, 12.5 per cent wheat bran, 6.1 per cent alfalfa meal, 6.1 per cent soybean oil meal, 3.1 per cent blackstrap molasses, 0.1 per cent salt, and 3.5 per cent polyethylene. The proximate analysis of this ration is presented in Table V.

Trial IV

Upon conclusion of the collection period of Trial III, the burros were transferred to the metabolism area and confined to the collection stalls. The burros were fed the ration they had received during Trial III. During the preliminary period four of the burros either refused to eat and drink in the collection stall or sustained rather serious injury due to constant kicking of the stall; therefore these animals were removed from the trial. As a result, feces collections were made from only four animals, two irradiated and two nonirradiated.

TABLE V

PROXIMATE ANALYSIS OF FEED USED IN METABOLISM TRIALS III AND IV (MOISTURE-FREE AND POLYETHYLENE-FREE[a] BASIS)

Nutrient	Percentage
Organic matter	92.9
Nitrogen-free extract	55.2
Crude protein	11.1
Crude fiber	25.1
Ether extract	1.4
Ash	7.1

[a]Polyethylene used for these trials was micothene polyethylene powder-M 710 (particle size of 50 mesh, density of 0.916, and medium molecular weight), U. S. Industrial Chemical Company, 99 Park Avenue, New York 19, New York.

Chemical Analyses

Fresh feces samples from these trials were dried at 60° C. for 72 hours. After allowing time for air equilibration, they were ground to pass through the medium mesh screen (2 mm.) of a Wiley Mill, subsampled, and placed in airtight glass jars. Chemical analyses for proximate nutrients in both feed and fecal samples were performed in accordance with A.O.A.C. (1960) methods. The procedure of Chandler, Kesler, and McCarthy (1964) was used for polyethylene analysis of the samples from Trial III.

Computation of Digestion Coefficients and Statistical Analysis

Nutrient digestion in Trials I, II, and IV was computed by subtracting the amount of nutrient excreted in the feces from the amount of nutrient ingested, and expressing the digestion coefficient as a percentage of the amount ingested. The digestion coefficients were calculated directly from the data in Trials I and II. In the case of Trial IV, however, all feed and fecal samples were first corrected to a polyethylene-free basis, because the polyethylene content of the feed was 3.5 per cent and up to 8.5 per cent in the feces. If not corrected, this relatively large percentage of indigestible material would have biased digestion coefficients downward (Chandler, Kesler, and Jones, 1966).

Feed and fecal samples from Trial III were also corrected for polyethylene content, and since this trial included an external indicator, the digestion coefficients were calculated with the following equation of Maynard and Loosli (1956):

$$\text{Digestibility} = 100 - \frac{\%\text{ indicator in feed}}{\%\text{ indicator in feces}} \times \frac{\%\text{ nutrient in feces}}{\%\text{ nutrient in feed}} \times 100.$$

Differences in digestion coefficients due to treatments were tested for statistical significance by the analysis of variance method (Steel and Torrie, 1960). In the case of crude fiber digestion, the differences between trial means for irradiated and nonirradiated burros were tested for statistical significance.

CHAPTER IV

RESULTS AND DISCUSSION

I. GROWTH STUDIES

Acute Effects of Irradiation

Even though the objectives of this study were concerned with the late effects of a sublethal dose of ionizing irradiation on the performance of growing burro foals, the acute effects of the irradiation must be evaluated to determine the degree of irradiation injury that was produced.

The growth curves of the irradiated and nonirradiated foals for the first 12 weeks postirradiation are presented in Figure 1. The greatest difference in weight between the two groups of foals occurred during the fifth week postirradiation; however, this difference was not statistically significant ($P>.10$). A loss of weight in burros exposed to $LD_{50(30)}$ levels of irradiation has been reported by Trum *et al.* (1959a) and in burros exposed to daily 200 R and 25 R fractionated doses of irradiation by Lane *et al.* (1955). Ziporin *et al.* (1959) presented data that indicated that the loss of weight in rats occurring after exposure to irradiation is due to anorexia. The foals in the present study were suckling their dams during the postirradiation period making it impractical to determine the amount of milk each foal consumed. However, no mammary swelling was observed in dams of irradiated foals which indicated that these foals consumed most of the milk produced.

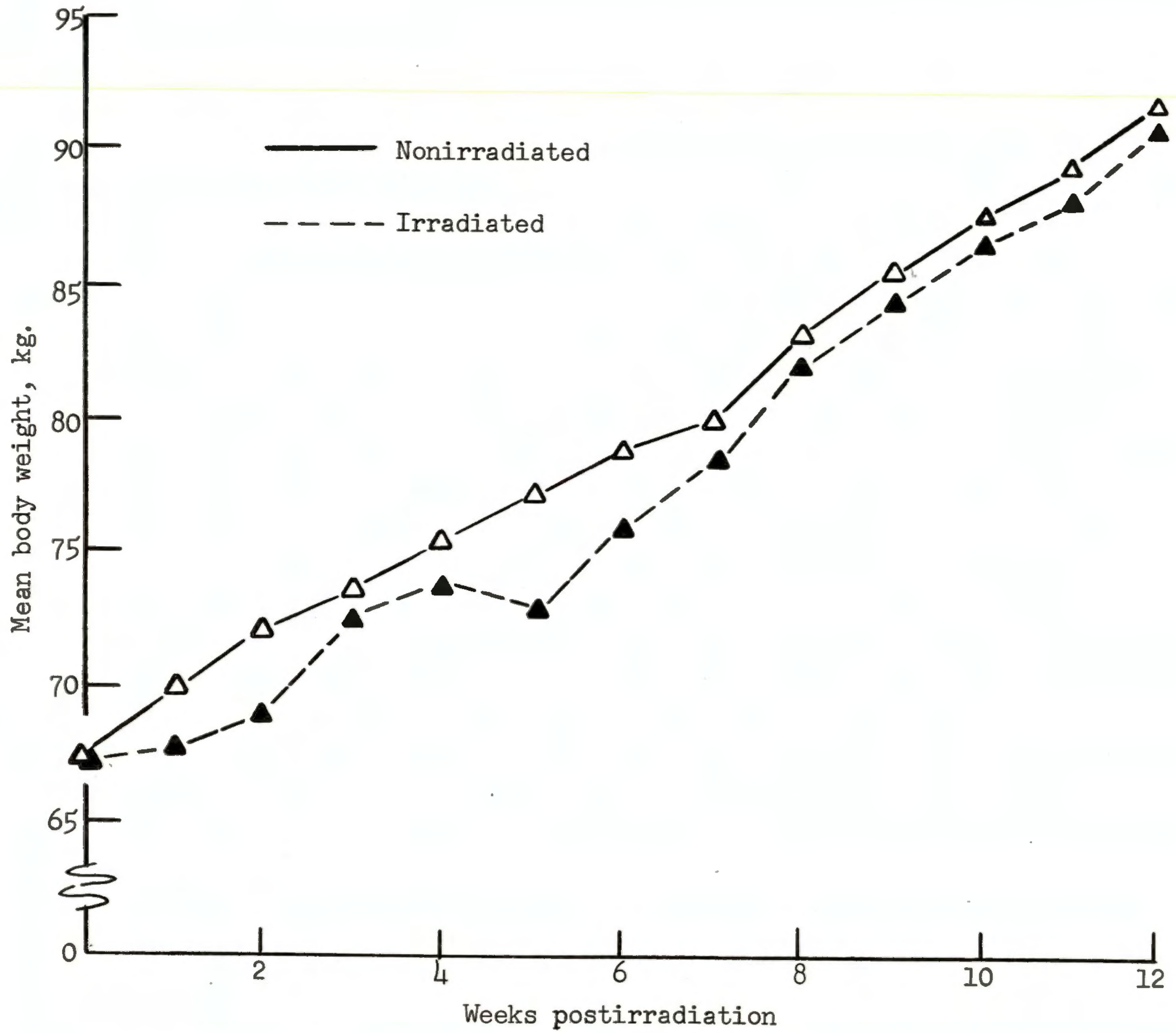

Figure 1. Growth curves of irradiated and nonirradiated foals for 12 weeks after irradiation.

The period between 3 and 5 weeks postirradiation was the most critical regarding the acute effects of the irradiation. In addition to the largest weight difference between irradiated and nonirradiated foals, a considerable amount of bloody mucus was excreted in the feces of the irradiated foals, several cases of bloody diarrhea were observed, and 2 of the 32 foals that were irradiated died from what was considered to be acute radiation effects. The most critical period for mature burros exposed to $LD_{50(30)}$ levels of irradiation was reported to be between the 22nd and 24th day following exposure (Trum et al., 1959a).

The high degree of sensitivity of the burro's blood system to whole-body irradiation is well documented (Rust et al., 1954b and 1955; Thomas and Brown, 1961). A graphic expression of the platelet and leukocyte populations of the irradiated foals involved in the present study is presented in Figure 2. The platelet and the leukocyte counts of the irradiated foals are expressed as a percentage of the counts obtained from the nonirradiated foals. As seen in Figure 2, the platelet population of the irradiated foals decreased to 22 per cent on the 15th day postirradiation and the leukocyte numbers decreased to 32 per cent by the 28th day after irradiation. Rust et al. (1954a) exposed mature burros to 200 R of gamma irradiation daily and reported that by the 14th day the blood platelet population decreased to 6 per cent of the control values just before death. The decline in leukocyte numbers observed in the present study is characteristic for burros that are exposed to both sublethal and lethal levels of irradiation (Rust et al., 1954b and 1955; Thomas and Brown, 1961).

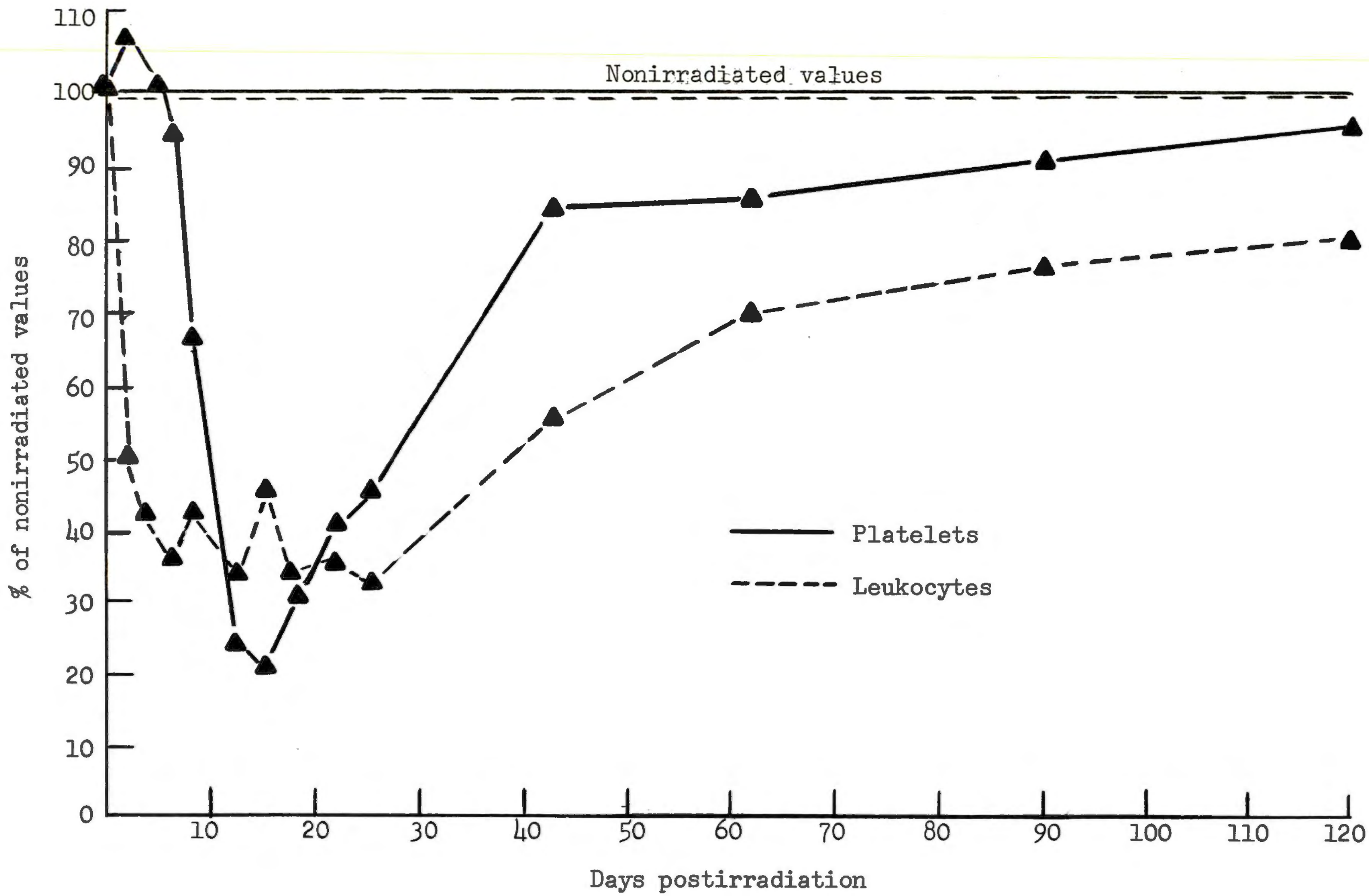

Figure 2. A graphic expression of platelet and leukocyte populations of the irradiated foals for 120 days postirradiation.

Total damage as a result of sublethal doses of irradiation cannot be measured quantitatively; however, the foregoing observations indicate that 250 R of ionizing whole-body irradiation was near the $LD_{50(30)}$ dose for a 120-day-old burro foal.

<u>Results Obtained During the Protein Feeding Trial</u>

The performance of each foal during the 364 days when rations containing the three levels of protein were fed is presented in Appendix Tables XIII and XIV. Tables XV and XVI in the Appendix are performance summaries of foals in the first and second replications, respectively. The performance of foals in both replications are pooled in Table VI. Obviously, irradiation had little or no detrimental effect on any of the performance traits tested. Nonirradiated foals gained weight somewhat more efficiently and grew slightly taller than their irradiated counterparts fed the same ration. As expected, the foals fed the ration containing 9 per cent crude protein did not grow as fast as the foals fed rations containing 14 or 18 per cent crude protein.

The analysis of variance of the pooled data for both replications is presented in Table VII. Table VIII contains the least squares constants for the pooled performance traits expressed as deviations from the overall mean for each trait.

<u>Total gain</u>. The growth curves for irradiated and nonirradiated foals fed rations containing the three protein levels are presented in Figure 3. These curves show that the greatest difference in the rate of gain was between foals fed the ration containing 9 per cent crude protein

TABLE VI

SUMMARY OF THE PERFORMANCE OF IRRADIATED AND NONIRRADIATED BURRO FOALS FED THREE LEVELS OF PROTEIN FOR 364 DAYS (BOTH REPLICATIONS)

Item	9% crude protein		14% crude protein		18% crude protein	
	Irradiated	Non-irradiated	Irradiated	Non-irradiated	Irradiated	Non-irradiated
No. on treatment	12	11	6	5	12	13
Weights, kg., av.						
Initial	88.6	91.1	93.5	90.5	92.8	92.5
Final	125.0	132.9	161.4	153.2	157.0	158.3
Gain	36.4	41.8	67.8	62.7	64.2	65.8
Av. daily gain	0.100	0.115	0.186	0.172	0.176	0.181
Feed consumed, kg., av.						
Total	770.7	779.2	1,256.1	1,145.5	1,205.4	1,207.1
Daily	2.12	2.14	3.45	3.15	3.31	3.32
Feed kg./kg. gain, av.	24.68	22.07	19.30	18.54	19.04	18.82
Growth						
Gain height, cm.	14.33	16.05	16.76	18.34	17.27	17.68
Gain heart girth, cm.	27.08	28.27	32.97	31.45	33.32	32.21

TABLE VII

ANALYSIS OF VARIANCE OF THE PERFORMANCE OF IRRADIATED AND NONIRRADIATED BURRO FOALS FED RATIONS CONTAINING THREE LEVELS OF PROTEIN

Source	Degrees of freedom	Mean squares				
		Total gain	Total feed	Feed/ unit gain	Increase in height	Increase in heart girth
Irradiation	1	49.53	7,755.15	31.14	25.41	0.00
Protein	2	4,493.31**	1,294,231.28**	168.39	37.67*	186.34**
Replication	1	932.93**	8,674.66	541.55**	25.54	144.09*
Sex	1	951.14**	42,859.40	255.61	29.47	94.36*
Sex x irradiation	1	146.40	5,225.27	0.01	18.19	7.87
Irradiation x protein	2	44.96	10,577.05	13.43	3.35	10.26
Residual	50	109.42	14,217.64	59.14	8.26	11.87

*P<.05.

**P<.01.

TABLE VIII

LEAST SQUARES CONSTANTS FOR PERFORMANCE TRAITS OF IRRADIATED AND NONIRRADIATED FOALS EXPRESSED AS DEVIATIONS FROM THE MEAN

Item	Total gain	Total feed	Feed/ unit gain	Increase in height	Increase in heart girth
Overall mean	54.80	1,038.19	20.60	16.59	30.66
Irradiation					
Nonirradiated	+0.99	-12.42	-0.79	+0.71	+0.01
250 R	-0.99	+12.42	+0.79	-0.71	-0.01
Protein					
9%	-15.82	-288.47	+1.94	-1.58	-3.56
14%	+4.84	+146.36	+1.27	+0.86	+2.13
18%	+10.98	+142.11	-3.21	+0.72	+1.43
Replication					
First	+4.44	+13.54	+3.38	+0.74	+1.75
Second	-4.44	-13.54	-3.38	-0.74	-1.75
Sex					
Male	+4.17	+27.78	-2.02	+0.74	+1.29
Female	-4.17	-27.78	+2.02	-0.74	-1.29
R^2	.683	.792	.274	.267	.508

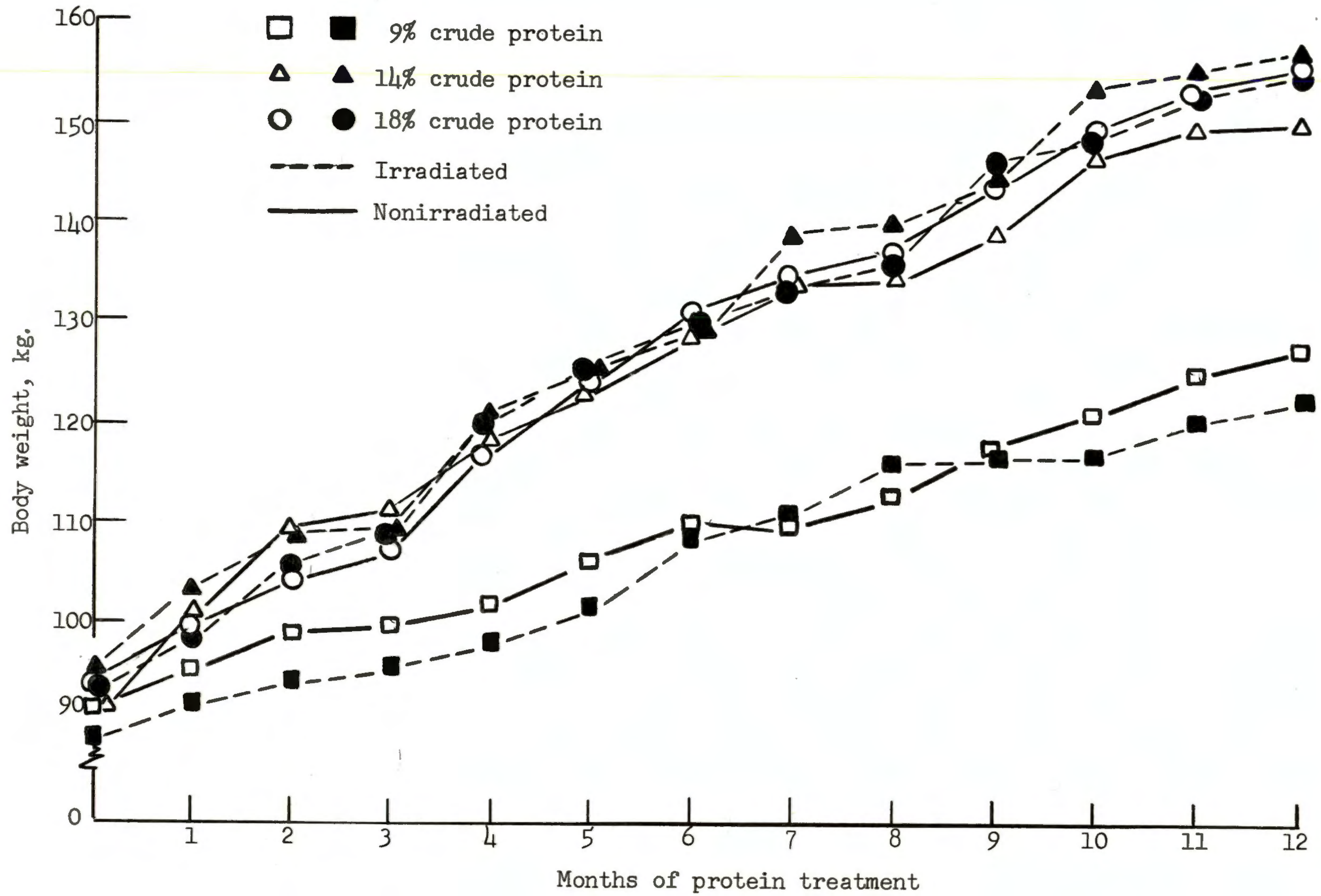

Figure 3. Growth curves of irradiated and nonirradiated burro foals fed three levels of protein.

and those fed rations containing 14 and 18 per cent crude protein. Statistical analysis of these data show a highly significant ($P<.01$) difference in rate of gain between the foals fed the rations containing 9 per cent crude protein and the ones fed at the 14 or 18 per cent levels. The difference in gain between foals fed the rations containing 14 and 18 per cent crude protein was not significant ($P>.10$).

The growth curves in Figure 3, page 46, also show quite vividly the very small effect of irradiation on the increase in body weight of the foals. Statistical analysis confirms that irradiation did not effect the growth of foals in this study ($P>.10$). There was a tendency in both replications for irradiated foals fed the ration containing 9 per cent crude protein to gain less weight than their nonirradiated counterparts (41.3 versus 48.5 and 31.5 versus 33.7 kg. for the first and second replications, respectively). On the other hand, during the first replication, irradiated foals fed rations containing 14 and 18 per cent crude protein gained slightly more weight than their nonirradiated counterparts (67.8 versus 62.7 and 69.1 versus 68.5 kg. for foals fed rations containing 14 and 18 per cent crude protein, respectively).

Since there were no significant differences in the gain of weight between irradiated and nonirradiated foals, these results would tend to confirm the findings of Goldwater and Entenman (1959) who reported no difference in the growth of irradiated and nonirradiated rats over a 26-week period while they were fed rations containing various sources of fat. The present results do not agree with the work of Carroll and Brauer (1961b) who reported a 15 per cent decrease in the growth of irradiated rats over a 25-week period. However, unlike the foals, the growth of both irradiated and nonirradiated rats was retarded for a considerable period of time before and after irradiation. In addition, the rats were exposed to a higher dose of irradiation.

The highly significant decrease ($P<.01$) in the amount of weight gained in the second replication (Table VII, page 44) may be associated with a severe case of dermatitis that appeared midway through the second replication and persisted until the trial was completed.

The difference in gain of body weight between the two sexes was highly significant ($P<.01$) in this study. Relatively little has been published regarding the growth patterns of the burro. Therefore, it was not possible to introduce factors into the statistical analysis that would have corrected growth data for sex.

As seen in Table VIII, page 45, the model used in this analysis accounted for almost 70 per cent ($R^2 = .683$) of the variation in total gain. It can be speculated that if all possible interactions were included in the model a higher percentage of the variation could have been explained. However, it is quite evident that the interactions omitted would not have contributed to the explanation of the main effects of irradiation.

<u>Total feed consumed</u>. The significantly lower gain in body weight observed for the foals fed the low protein ration can be attributed to the significantly lower ($P<.01$) amount of feed consumed by these animals. Overall, foals fed the ration containing 9 per cent crude protein

consumed 64 per cent as much feed as was consumed by the foals fed rations containing 14 or 18 per cent crude protein. These data agree with the work of Klosterman et al. (1956) who reported a reduction in appetite associated with beef cattle fed a ration containing a suboptimal level of protein.

Irradiation had no significant effect (P>.10) on the amount of feed the burros consumed. These data were calculated in order to obtain monthly averages for the purpose of determining if irradiation decreased appetite of the foals during the trial. Results of these calculations show that at no time during the 12-month period was the difference in feed consumption between irradiated and nonirradiated foals fed the same ration more than 8 per cent. In addition, it was not possible to detect any trends in the amount of feed consumed that could be attributed to irradiation. However, Carroll and Brauer (1961b) reported that the appetite of irradiated rats decreased after the seventh week of a 25-week test period.

The model used for analysis of these data accounted for almost 80 per cent (R^2 = .792) of the total variation in the amount of feed consumed, even though the level of protein fed was the only independent variable included in the model that had a significant effect.

Feed efficiency. The analysis of variance presented in Table VII, page 44, shows that the difference in feed efficiency between replications was highly significant (P<.01). This difference, no doubt, can be attributed to the dermatitis that infected the foals in the second replication during the last half of that trial.

The nonsignificant (P>.10) effect of crude protein level of the ration that was observed in the present study is in agreement with work of Winchester, Hiner, and Scarborough (1957) who reported similar efficiency in feed utilization in co-twin cattle fed rations containing high and low levels of crude protein.

Irradiation had no significant effect (P>.10) on feed efficiency of the burros in this study. Goldwater and Entenman (1959) reported that irradiated rats were 20 per cent less efficient in feed conversion than their nonirradiated counterparts. Inspection of the present data shows that, in general, there was a consistent trend for nonirradiated foals to be more efficient in feed utilization than the irradiated foals fed the same level of crude protein. The difference in feed efficiency between irradiated and nonirradiated foals was greatest (11.8 per cent) for foals fed the low level of crude protein, intermediate (4.1 per cent) for those fed the intermediate level, and smallest (0.8 per cent) for those fed the high level. This inverse relationship between crude protein level of the feed and difference in feed efficiency between irradiated and nonirradiated foals was apparent in both replications. However, it was more pronounced in the first replication where the difference in feed efficiency between the two groups of foals was 22.8, 4.1, and 0.7 per cent for foals fed rations containing 9, 14, and 18 per cent crude protein, respectively. The difference in feed utilization for irradiated and nonirradiated foals in the second replication was 1.3 and 0.3 per cent for those fed the 9 and 18 per cent levels of crude protein, respectively. This difference in variation between the two trials can be attributed to the previously mentioned dermatitis infection. This pathological condition apparently decreased the feed efficiency of both irradiated and nonirradiated foals which tended to mask any difference that occurred as a result of irradiation. The 22.8 per cent decrease in feed efficiency that was observed as a result of irradiation in foals fed the low level of crude protein during the first replication was in close agreement with the 20 per cent decrease observed in irradiated rats by Goldwater and Entenman (1959).

The statistical model that was used for this analysis accounted for less than 30 per cent (R^2 = .274) of the total variation associated with feed efficiency. This is considerably less than the coefficients of determination of .683 and .792 that were calculated for total gain and total feed consumed, respectively. These data indicate that independent variables other than the one considered important in this study have a pronounced effect on this trait. Possible variables that could have a considerable effect on feed efficiency may be genetic and environmental in nature.

Increase in height and increase in heart girth. The analysis of variance which appears in Table VII, page 44, shows that the level of protein in the ration had a highly significant effect ($P<.01$) on increase in heart girth and a significant ($P<.05$) effect on increase in height. In addition, there was a significant ($P<.05$) difference on increase of heart girth due to replication and sex.

These results are quite reasonable when one considers that the heart girth measurement of the burro could include some muscular growth, whereas the height at the point of the shoulders does not include an appreciable amount of muscle. With these considerations, it is reasonable to expect that a low plane of nutrition would have a greater effect on a measurement that includes muscular growth (heart girth) than on a measurement that includes mostly skeletal growth (height). Sex, environmental, and pathological changes would have little effect on skeletal growth *per se*, whereas the amount of muscling could be altered by these factors. A comparison of the coefficients of determination (Table VIII, page 45) associated with increase in height (.267) and increase in heart girth (.508) also indicate that increase in height is associated with skeletal growth more so than increase in heart girth. The statistical model used to evaluate these data did not include variables associated with genetic effects. Since skeletal growth is associated with genetic potential, the amount of variation in the increase of height that was accounted for is relatively low (26.7 per cent). In the case of increase in heart girth the amount of variation accounted for by this model was somewhat higher (50.8 per cent) because muscular growth was effected by the independent variables that were included in the model.

The differences of both increase in height and heart girth between irradiated and nonirradiated foals in this study were not statistically significant ($P>.10$). This observation is in contrast with various reports dealing with children that were exposed to atomic bomb blasts. However, exposure to a bomb is instantaneous and at least part of the difference between the present work and the following reports can be explained on this basis. Greulich, Crismon, and Turner (1953) reported that the retardation in the physical growth and development of children who survived the atomic bomb blasts of Hiroshima and Nagasaki was evident 5-1/2 years after the bombing. In general, the boys tended to be more retarded than girls and the most pronounced retardation occurred in children who were between 12 and 18 months of age at the time of the bombing. Similar observations were made by Sutow, Conard, and Griffith (1965) who studied 38 children that were exposed to fallout irradiation in the Marshall Islands. Wood *et al.* (1966) made an extensive study of Japanese teenagers who were exposed to the bomb blasts in utero and they reported an inverse relationship between the size of the individuals and the distance their mothers were from the center of the blasts.

Power of the test. The power of the statistical test to distinguish a real difference of the magnitude equal to the observed difference between irradiated and nonirradiated foals in all traits studied was negligible.

Nutritional Aspects

Squibb (1958) and Cunha (1966) presented review articles dealing with horse nutrition and both authors stressed a need for additional research in this area. Although the present work was not specifically designed to determine nutrient requirements, the design of the experiment was such that inferences can be made concerning the protein requirements of the growing burro foal.

Obviously, the ration that contained 9 per cent crude protein supplied a suboptimal level of this nutrient (Figure 3, page 46).

The protein content of this ration was considerably below the levels recommended by Morrison (1956) and N.R.C. (1966) for growing horse foals. Data collected during the second replication was not used for this analysis since the ration containing 14 per cent crude protein was not fed. The average daily feed consumption during the 364-day period was 3.30 and 3.26 kg. for the foals fed the rations containing 14 and 18 per cent crude protein, respectively. However, the foals fed the 18 per cent level of protein consumed 27 per cent more protein than those fed the 14 per cent level (0.59 _versus_ 0.46 kg. per day). The foals fed the ration containing 18 per cent crude protein gained 68.8 kg. and had a feed efficiency ratio of 17.4 kg. of feed per kg. of gain, while those fed the 14 per cent level of crude protein gained 65.2 kg. with a feed efficiency ratio of 18.9 kg. This difference between the two groups was nonsignificant ($P>.10$). On the basis of these data, it appears that a ration containing 14 per cent crude protein is adequate for maximum growth in the burro foal. This observation is in close agreement with the N.R.C. (1966) requirement of 13.1 per cent that is recommended for a 91 kg. horse foal whose mature weight would be 273 kg., particularly if one considers that nutritional requirements per unit weight decrease as the weight of the animal increases. Morrison (1956) recommends a 11.5 per cent level of digestible protein for the 91 kg. horse foal. Based on digestion coefficients published for the horse by Olsson and Ruudvere (1955) and for the burro by Knapka _et al._ (1967), this would approach a 15 per cent crude protein level in the ration.

On the basis of the present data, Morrison's (1956) recommendations for the growing horse would appear to be slightly higher than is required for the growing burro; however, additional data concerning the nutrient requirements of burros are required to varify this observation.

The Period Following the Protein Trial

The objective of collecting data during this period was to detect possible differences in the ability of irradiated and nonirradiated foals to recover from the nutritional stress. The criteria of evaluation were gain in weight during the first 76 days of this period and gain in weight, increase in height, and increase in heart girth over the 152 days.

It was not possible to obtain a valid estimate of the amount of hay each animal consumed because hay was fed _ad libitum_ to groups of individuals that varied in weight. During the 152-day period, irradiated foals consumed 3.69 kg. of concentrate per day as compared to 3.67 kg. consumed by the nonirradiated foals.

The analysis of variance for the performance of the foals during the postprotein period is presented in Table IX and the least squares constants for each of the traits evaluated are shown in Table X. The difference of increase in height between irradiated and nonirradiated foals was highly significant ($P<.01$). This phenomenon is difficult to explain on the basis of the present data, particularly since the irradiated foals increased more in height than the nonirradiated. An increase in body size as a result of radiation is contrary to the reports of Greulich, Crismon, and Turner (1953) and Sutow, Conard, and Griffith

TABLE IX

ANALYSIS OF VARIANCE FOR THE PERFORMANCE OF IRRADIATED AND NONIRRADIATED FOALS DURING THE 152 DAYS FOLLOWING THE PROTEIN FEEDING TRIAL

		Mean squares			
Source	d. f.	Gain 76 days postprotein	Gain 152 days postprotein	Increase in height (152 days)	Increase in heart girth (152 days)
Irradiation	1	0.83	8.92	27.93**	1.01
Protein[a]	2	59.16	949.82**	55.25**	90.60**
Replication	1	1,954.55**	1,555.11**	26.54*	136.33**
Sex	1	2.99	136.69	14.26	55.12**
Sex x irradiation	1	8.33	0.74	12.81	1.08
Irradiation x protein	2	25.25	5.88	3.59	3.78
Residual	42	23.43	43.42	5.23	7.15

[a]These data were classified on the basis of the level of protein that the foals were fed during the protein trial.

*P<.05.

**P<.01.

TABLE X

LEAST SQUARES CONSTANTS FOR PERFORMANCE TRAITS OF IRRADIATED AND NONIRRADIATED FOALS DURING THE 156 DAYS FOLLOWING THE PROTEIN FEEDING TRIAL

Item	Gain 76 days postprotein	Gain 152 days postprotein	Increase in height (152 days)	Increase in heart girth (152 days)
Overall mean	13.42	15.47	3.15	3.28
Irradiation				
Nonirradiated	+0.14	+0.48	-0.84	+0.15
250 R	-0.14	-0.48	+0.84	-0.15
Protein				
9%	+2.33	+8.46	+2.26	+2.95
14%	-1.69	-3.66	-1.73	-2.77
18%	-0.64	-4.80	-0.53	-0.18
Replication				
First	+6.92	+6.18	-0.81	-1.83
Second	-6.92	-6.18	+0.81	+1.83
Sex				
Male	-0.25	-1.69	-0.56	-1.07
Female	+0.25	+1.69	+0.56	+1.07
R^2	.784	.716	.439	.515

(1956) who reported that the growth of children was retarded as a result of exposure to atomic bomb blasts. Carroll, Phillips, and Kimeldorf (1966) presented data showing that X irradiation inhibited the growth of limb bones of the rat. The significant difference in the increase of height between irradiated and nonirradiated foals could very well be an artifact because this is the only trait that was significantly different as a result of radiation throughout this study and the difference between the two groups of foals was only 1.68 cm. Therefore, additional data are required before any positive conclusion can be reached regarding this difference.

During the first 76 days of the postprotein period there were no significant differences ($P>.10$) in the amount of weight the foals gained that could be attributed to the level of crude protein that they had previously consumed (Table IX, page 56). However, during the entire 152 days the foals that had previously consumed the ration containing 9 per cent crude protein gained significantly ($P<.01$) more weight than the ones that consumed the 14 and 18 per cent levels of crude protein (Table IX, page 56). The difference in gain between the latter two groups was not significant ($P>.10$). The tendency for all foals to gain an equal amount of weight during the first weeks of the postportein period can be attributed to the small amount of concentrate that was fed in order to prevent gastric disorders as a result of overeating. Each foal was fed 0.9 kg. of the concentrate per day for the first 3 days and the amount was gradually increased until the 30th day when ad libitum feeding was initiated.

The significant increase (P<.01) in the performance of foals that had previously consumed the ration containing 9 per cent crude protein would be expected on the basis of compensatory gains described in beef cattle by Castle, Wallace, and Bogart (1961). The amount of concentrate consumed by the foals during this period was 3.74, 3.51, and 3.76 kg. per day for the foals that previously consumed the rations containing 9, 14, and 18 per cent crude protein, respectively.

The significant (P<.01) decrease in the performance of foals during the second replication was consistent throughout this entire study, and, as mentioned earlier, this difference may be attributed to the infectious dermatitis.

The model used for this analysis accounted for a relatively high percentage of the variation that was associated with the various traits as noted by the coefficients of determinations in Table X, page 57.

These data indicate that radiation had no significant effect on the performance of the foals during the postprotein period and that the burro reacts to periods of suboptimal nutrition similar to other species in that compensatory gains were observed.

II. DIGESTION TRIALS

Trial I

This was a total-collection trial that was designed to determine possible effects of age on nutrient digestion in the burro. The results presented in Table XI (see Table XVII in the Appendix for individual digestion coefficients) indicate that 3 years difference in age between

TABLE XI

APPARENT DIGESTION COEFFICIENTS FROM BURROS OF TWO AGE GROUPS (TRIAL I)

	Nutrient				
Burros	Organic matter	Crude protein	Ether extract	Crude fiber	N.F.E.
2-year olds[a]	71.39	83.70	69.63	29.44	76.02
C.V.[b]	1.90	3.16	2.85	20.30	1.53
5-year olds	70.91	82.33	67.85	27.17	76.22
C.V.	1.86	4.14	7.56	14.08	1.43

[a]Each digestion coefficient is the mean of five values.

[b]Coefficients of variation.

these two groups of burros had little effect on the apparent digestibility of the various nutrients. The differences in the digestion coefficients between the two age groups were not significant (P>.10). However, the digestion of all nutrients, with the exception of nitrogen-free extract digestion, was higher in the 2-year-old burros.

The large coefficient of variation that was associated with crude fiber digestion is apparently characteristic of equine animals. Fonnesbeck (1965) noted a considerable amount of variation in the digestibility of cellulose among horses and indicated that the magnitude of digestion is characteristic of each horse.

Digestion coefficients for all nutrients obtained from this trial were in close agreement with the digestion coefficients reported by Olsson and Ruudvere (1955) for horses that were fed rations containing similar amounts of crude fiber as the present experimental ration.

Trials II, III, and IV

The digestion coefficients obtained from the trials that compared the digestion of irradiated and nonirradiated burros within each trial are presented in Table XII (see Table XVIII in the Appendix for individual digestion coefficients). Statistical analysis of these data indicated that there were no significant differences in nutrient digestion between irradiated and nonirradiated burros (P>.10).

Because crude fiber digestion was consistently lower for irradiated burros throughout the three trials, the digestion coefficients for this nutrient from the three trials were pooled and tested for a significant treatment difference. Results of this analysis also indicated that

TABLE XII

APPARENT DIGESTION COEFFICIENTS FROM IRRADIATED AND NONIRRADIATED BURROS

Burros	Nutrient: Organic matter	Crude protein	Ether extract	Crude fiber	N.F.E.
		Trial II			
Nonirradiated[b]	70.82	83.41	66.23	24.96	76.36
C.V.[a]	1.24	0.46	3.93	20.02	0.83
Irradiated	70.77	83.80	66.79	22.80	75.17
C.V.	1.82	2.63	6.27	19.29	6.15
		Trial III			
Nonirradiated[c]	65.00	70.09	37.32	32.10	75.57
C.V.	6.96	3.61	11.63	25.38	5.47
Irradiated	61.09	64.65	38.81	25.61	73.62
C.V.	7.35	5.84	15.65	30.77	5.19
		Trial IV			
Nonirradiated[d]	65.19	64.39	48.46	31.99	76.75
C.V.	3.51	5.05	14.62	9.35	3.24
Irradiated	63.96	63.88	45.13	28.79	76.32
C.V.	0.41	1.59	28.08	0.00	0.00

[a]Coefficients of variation.

[b]Each digestion coefficient is the mean of five values in Trial II.

[c]Each digestion coefficient is the mean of four values in Trial III.

[d]Each digestion coefficient is the mean of two values in Trial IV.

irradiation did not significantly effect crude fiber digestion ($P > .10$).

The digestion of must nutrients was somewhat lower in Trials III and IV than in the first two trials. This lower nutrient digestion may be associated with the higher level of crude fiber that was consumed by the burros in the last two trials (11.3 versus 25.1 per cent).

The somewhat higher coefficients of variation that were observed in Trial III as compared to Trials II and IV may be explained on the basis that the burros in Trial III were not confined to metabolism crates and differences in physical activity between burros could have contributed to greater individual variation in nutrient digestion. In contrast, Trials I, II, and IV were total-collection trials and burros were limited to a similar amount of physical activity which resulted in less individual variation of nutrient digestion.

Any differences in ether extract digestion or coefficients of variation associated with ether extract digestion would be of little consequence in these studies, since the rations contained small amounts of fat and metabolic sources of this nutrient could have markedly affected apparent digestibility.

The lower protein digestibility that occurred in Trials III and IV as opposed to Trial II is probably associated with the crude protein content of the experimental rations (the rations used in Trial II and Trials III and IV contained 16.8 and 11.1 per cent crude protein, respectively). Holter and Reid (1959) summarized data obtained from cattle and sheep and reported that the apparent digestibility of protein increases as the concentration of crude protein in forages increases.

These data indicate that irradiation did not effect nutrient digestion in any of the burros involved in these trials. However, all animals used in these trials were in good health. It is possible that alterations in nutrient digestion would be detected if digestion trials were conducted with burros that were in the process of losing weight (Brown, Johnson, and Cross, 1965) after having survived radiation for an extended period of time.

CHAPTER V

SUMMARY

Experiments were conducted to determine possible late effects of sublethal levels of whole-body gamma radiation on growth of burro foals and on nutrient digestion in burros that had survived radiation for as long as 7 years.

A growth study was replicated and included 32 burro foals that had been exposed to 250 R ^{60}Co gamma radiation during their fourth month of life and 31 nonirradiated foals. The foals were weaned during their seventh month of life (90 days postirradiation) and allotted into three groups based on radiation treatment, weight, sex, size, and radiation history of their dams. Each group of foals was then randomly selected to be fed a ration containing either 9, 14, or 18 per cent crude protein during a 364-day feedlot trial. Criteria used to evaluate the treatments included body weight gains, feed consumption, feed efficiency, increase in height, and increase in heart girth. Least squares analysis of these data indicated that radiation had no significant effect ($P>.10$) on any of the traits tested. There was a consistent though nonsignificant ($P>.10$) trend for irradiated foals to be less efficient in feed utilization than their nonirradiated counterparts.

With the exception of feed efficiency, foals that consumed the ration containing 9 per cent crude protein performed at a significantly ($P<.01$) lower level than the foals fed rations containing 14 or 18 per

cent crude protein. The differences in the performance between foals fed the 14 and 18 per cent levels or crude protein were not significant (P>.10).

During the 152 days following the end of the protein feeding trial, irradiated foals increased in height significantly more (P<.01) than nonirradiated foals. This difference cannot be explained on the basis of the present data. However, differences in gain of body weight and increase in heart girth were not significant (P>.10).

In four digestion trials a total of 21 nonirradiated and 11 irradiated mature burros were used. The first trial was designed to determine the effect of age on nutrient digestion. Results indicated that 3 years difference in age did not effect digestion of organic matter, crude protein, ether extract, crude fiber, or nitrogen-free extract.

The results of the three trials that involved irradiated burros indicated that there were no significant (P>.10) differences in nutrient digestion as a result of irradiation. However, there was a consistent trend for irradiated burros to digest less crude fiber than nonirradiated burros.

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APPENDIX

TABLE XIII

INDIVIDUAL PERFORMANCE OF FOALS DURING THE PROTEIN FEEDING TRIAL (FIRST REPLICATION)

Foal no.	Initial weight (kg.)	Gain in body weight (kg.)	Total feed consumed (kg.)	Increase in height (cm.)	Increase in heart girth (cm.)
		Irradiated foals fed 9% protein ration			
6300	88.6	25.0	810.0	11.7	21.3
6305	79.5	43.2	719.1	13.7	25.4
6312	88.6	47.7	841.8	17.5	22.9
6325	104.5	54.5	858.2	18.4	29.2
6328	77.3	36.4	685.9	15.2	21.0
6336	72.7	40.9	715.0	13.0	31.4
		Nonirradiated foals fed 9% protein ration			
6311	97.7	61.4	867.7	17.5	25.7
6313	93.2	43.2	770.9	16.2	26.0
6317	77.3	54.5	789.1	17.5	33.0
6330	84.1	38.6	685.9	14.9	25.7
6338	84.1	43.2	720.0	16.8	27.3
6339	93.2	50.0	792.7	17.1	27.3
		Irradiated foals fed 14% protein ration			
6301	109.1	50.0	1,241.8	14.9	29.8
6308	95.4	77.3	1,341.4	17.1	33.7
6309	90.9	84.1	1,390.0	17.8	37.5
6324	95.4	68.2	1,338.6	16.9	33.7
6329	93.2	52.3	990.9	14.3	28.6
6333	77.3	75.0	1,234.1	19.7	34.6
		Nonirradiated foals fed 14% protein ration			
6302	102.3	52.3	1,091.4	16.2	27.6
6315	90.9	54.5	1,124.1	17.8	28.9
6319	106.8	61.4	1,188.2	19.7	36.8
6321	68.2	70.4	1,029.5	19.4	32.4
6332	84.1	75.0	1,294.5	18.7	31.4

TABLE XIII (continued)

Foal no.	Initial weight (kg.)	Gain in body weight (kg.)	Total feed consumed (kg.)	Increase in height (cm.)	Increase in heart girth (cm.)
		Irradiated foals fed 18% protein ration			
6306	100.0	81.8	1,330.9	21.3	34.6
6307	113.6	68.2	1,302.2	15.2	26.7
6323	88.6	56.8	1,012.3	13.7	31.8
6326	77.3	72.7	1,161.8	11.1	32.4
6334	93.2	65.9	1,186.4	19.4	28.6
		Nonirradiated foals fed 18% protein ration			
6310	93.2	63.6	1,205.4	16.2	29.5
6314	95.4	59.1	1,212.3	18.1	30.5
6316	86.4	81.8	1,310.9	22.6	27.9
6322	113.6	77.3	1,289.5	20.6	35.9
6331	72.7	72.7	972.3	18.1	32.7
6337	86.4	56.8	1,044.5	8.6	24.4

TABLE XIV

INDIVIDUAL PERFORMANCE OF FOALS DURING THE PROTEIN FEEDING TRIAL (SECOND REPLICATION)

Foal no.	Initial weight (kg.)	Gain in body weight (kg.)	Total feed consumed (kg.)	Increase in height (cm.)	Increase in heart girth (cm.)
Irradiated foals fed 9% protein ration					
6405	103.6	32.7	871.9	7.6	29.2
6408	73.6	35.4	655.4	15.5	27.9
6411	98.6	44.5	880.9	15.9	31.1
6419	93.2	45.4	870.0	16.8	33.0
6420	94.1	10.4	595.9	13.0	23.5
6422	84.1	20.4	744.5	13.7	28.9
Nonirradiated foals fed 9% protein ration					
6404	110.4	41.8	843.2	15.6	28.9
6409	95.4	40.9	912.7	16.8	29.8
6410	91.8	10.4	652.3	12.4	26.4
6416	94.1	33.2	737.5	14.6	31.4
6423	80.4	42.3	796.8	17.1	29.2
Irradiated foals fed 18% protein ration					
6400	110.0	71.8	1,472.7	15.9	38.1
6402	106.8	46.8	1,195.0	14.0	28.9
6406	81.8	60.0	1,223.6	16.8	39.4
6414	94.5	68.2	1,384.5	18.4	31.1
6417	100.0	50.9	1,096.4	18.1	29.8
6425	71.4	65.0	1,041.4	22.9	41.3
6426	76.4	62.3	1,056.4	20.6	37.5
Nonirradiated foals fed 18% protein ration					
6401	106.8	52.3	1,187.3	15.6	33.0
6403	96.8	71.4	1,318.2	18.7	35.6
6407	103.6	78.2	1,443.6	21.3	40.6
6413	91.8	44.5	1,101.8	13.3	29.2
6415	96.4	61.8	1,201.8	17.1	33.0
6418	79.5	50.0	1,048.6	16.8	28.9
6424	80.4	85.4	1,364.1	22.9	37.5

TABLE XV

SUMMARY OF PERFORMANCE FOR IRRADIATED AND NONIRRADIATED BURRO FOALS FED VARIOUS LEVELS OF DIETARY PROTEIN FOR 364 DAYS (FIRST REPLICATION)

Item	9% crude protein		14% crude protein		18% crude protein	
	Irradiated	Non-irradiated	Irradiated	Non-irradiated	Irradiated	Non-irradiated
No. on treatment	6	6	6	5	5	6
Weights, kg., av.						
Initial	85.2	88.3	93.5	90.5	94.5	91.3
Final	126.5	136.7	161.4	153.2	163.6	159.9
Gain	41.3	48.5	67.8	62.7	69.1	68.5
Av. daily gain	0.113	0.133	0.186	0.172	0.190	0.188
Feed consumed, kg., av.						
Total	771.6	771.0	125.6	1,145.5	1,198.9	1,172.5
Daily	2.12	2.12	3.45	3.15	3.29	3.22
Feed gain, kg., av.	19.79	16.12	19.30	18.54	17.44	17.32
Growth, cm.						
Gain height	14.94	16.62	16.76	18.34	16.13	17.35
Gain heart girth	25.20	27.51	32.97	31.45	30.78	30.15

TABLE XVI

SUMMARY OF PERFORMANCE FOR IRRADIATED AND NONIRRADIATED BURRO FOALS FED VARIOUS LEVELS OF DIETARY PROTEIN FOR 364 DAYS (SECOND REPLICATION)

Item	9% crude protein		18% crude protein	
	Irradiated	Non-irradiated	Irradiated	Non-irradiated
No. of treatment	6	5	7	7
Weights, kg., av.				
Initial	92.0	94.5	91.5	93.6
Final	123.5	128.2	152.3	157.0
Gain	31.5	33.7	60.7	63.4
Av. daily gain	0.086	0.092	0.168	0.174
Feed consumed, kg., av.				
Total	769.8	788.9	1,210.0	1,237.9
Daily	2.11	2.17	3.32	3.40
Feed gain, kg., av.	29.58	29.20	20.18	20.11
Growth, cm.				
Gain height	13.77	15.32	18.08	17.96
Gain heart girth	28.95	29.16	35.15	33.99

TABLE XVII

INDIVIDUAL APPARENT DIGESTION COEFFICIENTS FROM BURROS OF TWO AGE GROUPS (TRIAL I)

Burro no.	Nutrient				
	Organic matter	Crude protein	Ether extract	Crude fiber	N.F.E.
			2-year olds		
118	69.77	79.88	71.92	22.89	75.71
103	71.60	84.63	70.39	26.53	76.60
106	70.42	84.68	67.78	29.21	74.40
120	71.94	86.86	70.75	29.75	75.85
101	73.24	82.45	67.33	38.80	77.55
			5-year olds		
701	69.99	79.75	73.18	24.23	75.79
702	69.38	78.58	60.67	27.01	75.38
500	70.80	85.87	64.63	27.12	75.23
507	71.69	85.87	69.40	24.04	76.92
518	72.69	81.57	71.83	33.47	77.76

TABLE XVIII

INDIVIDUAL APPARENT DIGESTION COEFFICIENTS FROM IRRADIATED AND NONIRRADIATED BURROS

Burro no.	Nutrient				
	Organic matter	Crude protein	Ether extract	Crude fiber	N.F.E.
		Trial II (nonirradiated)			
427	71.13	83.06	64.25	28.77	76.30
439	71.79	84.07	69.23	28.66	76.77
437	71.09	83.25	68.42	22.15	77.21
A47	70.68	83.27	63.17	27.76	75.79
A40	69.43	83.41	66.06	17.48	75.74
		Trial II (irradiated)			
432	72.62	83.82	73.21	28.69	77.89
412	70.58	82.29	65.07	25.64	76.23
A58	71.45	87.26	65.61	22.25	71.65
426	69.64	84.08	61.09	18.07	75.79
436	69.56	81.56	68.96	19.34	75.82
		Trial III (nonirradiated)			
321	70.21	73.63	41.45	43.90	77.95
314	59.29	69.40	31.28	25.58	69.44
316	64.37	67.30	39.05	28.14	76.76
331	66.13	69.71	37.50	30.80	78.14
		Trial III (irradiated)			
308	62.93	64.56	42.05	25.13	76.90
301	57.39	63.01	46.58	16.81	71.41
309	57.46	61.12	32.00	23.21	69.38
326	66.59	69.90	34.61	35.47	76.81
		Trial IV (nonirradiated)			
321	65.74	68.99	58.57	30.09	77.72
316	64.68	59.79	38.35	33.89	75.78
		Trial IV (irradiated)			
309	64.16	63.16	54.09	28.80	76.52
306	63.77	64.06	36.17	28.79	76.11