ABSTRACT

THE EFFECT OF SHORT-TERM FEEDING OF CATHODE RAY- AND GAMMA-IRRADIATED WHEAT UPON FAT DEPOSITION AND ENZYME SYSTEMS IN THE RAT LIVER

by Norma Magnus Gilmore

Ionizing radiation has been hailed as a most effective means of decreasing food storage losses through better insect control. Although small chemical changes are known to occur with radiation, foods so preserved have yet to be shown detrimental to animals with regard to reproduction, longevity, and growth. Wheat is one of the basic grains capable of long term storage and irradiation could conceivably increase its storage ability. Any process which may affect the future utilization of such wheat needs investigation. This nutritional study was undertaken to further evaluate the effects of feeding four levels each of cathode ray- and gamma-irradiated wheat to experimental animals for 28 days. Growth measurements and protein efficiency ratios were examined. To investigate possible micro alterations which the metabolizing of an irradiated food may produce, liver enzyme systems xanthine oxidase and cytochrome oxidase were measured and histological sections were made of the livers. Total liver nitrogen and fat were also measured.

There appeared to be no difference in the feed intakes and weight gains due to the type of irradiation, but there was indication

that the level of irradiation had some effect. The animals tended to grow at the same rate except for those on the highest level of irradiation who appeared to grow somewhat more slowly. The same trend persisted when the average weight gains per gram of diet eaten were calculated. Protein efficiency ratios were found to be similar in animals fed the lower levels of irradiation but again, the highest level of irradiation, regardless of the type incorporated into diets, resulted in lower protein efficiency ratios.

The activities of xanthine oxidase and cytochrome oxidase measured in the livers of animals fed irradiated diets were consistently lower than those obtained from the livers of control wheat-fed animals. The depression of activity of both enzyme systems was most pronounced in livers from gamma-irradiated wheat-fed animals. Histological examination of fat deposition within the cell and lobule of the individual livers presented a picture of possible intensified dietary unbalance, especially in the gamma-irradiated wheat-fed animals. No significant alterations were found in the total liver nitrogen or fat determinations.

The findings suggest that the type of radiation may have more significant influence than the level of irradiation.

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By

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INTRODUCTION

Preservation of food has been an age-old problem for man and until recently has been limited more or less to the following methods as given by Tanner ('44):

1. Asepsis.
2. Addition of chemical substances: sodium benzoate, salt or sugar, or both, spices.
3. Low temperatures: refrigeration, slow-freezing, and quick freezing.
4. High temperatures: pasteurization, boiling, and canning.
5. Fermentation: sauerkraut, pickles, and fermented milks.
6. Abstraction of moisture: meats, vegetables, and fruits.

With the advent of atomic power and the huge stockpiling of atomic waste products, great interest has been generated in using atomic waste products for food preservation. Ionizing radiation has been hailed as a most effective means of sterilization, a means of decreasing food losses because of better insect control, a means of improving flavor and texture of meats, fruits, and vegetables since no heat is required, a means of providing increased variety of food selection and distribution of perishables, and as a means of extending shelf-life. (Peterman, '56; Ryer, '56).

A most desirable quality afforded by irradiation, especially with these foods capable of long term storage, is the control of insect and mold infestation. The greatest problem in the storage of grain

is fungal growth which is the major source of respiration and heating. In time, respiration and heating will lead to chemical deterioration of the grain. Yen, Milner, and Ward ('56) noted that when the moisture content of wheat was maintained at 12% or 20%, gamma dosages of 250,000 and 625,000 rep, respectively, would eliminate grain spoilage fungi but below these levels molds would proliferate. The effect of irradiation upon bacterial contamination has been studied also, and radiosensitivity appears to vary from species to species. Numerous investigations have generally suggested the following most desirable levels of irradiation applicable to commercial use:

> 12,000 rep, potatoes and onions will be inhibited from sprouting;
>
> 10,000 to 20,000 rep, adult insects will be sterilized;
>
> 25,000 rep will be required to kill worms, flukes, and many insects;
>
> 200,000 rep will be the pasteurization level at which most micro-organisms will be killed;
>
> 250,000 to 625,000 rep, grain will be preserved from fungal growth;
>
> and 2,000,000 rep the sterilization level at which the most resistant of bacteria will be killed.*

In 1954, Lehman and Lang made the first statement concerning ionizing radiation in food preservation as viewed by the Food and Drug Administration. The authors advocated a conservative approach because of the inadequate means of testing safety. It was suggested that the minimum dosage of cathode ray and gamma-irradiation necessary to effect

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*rep= roentgen equivalent physical and is equivalent to the absorption of 93 ergs per unit gram of density.

commercially satisfactory sterilization be determined first; then, the minimum dosage which would produce definite deterioration of a product. And finally, somewhere between these two extremes, the minimum dosage for processing food which would be acceptable in texture, color, odor, smell, taste, nutritive value, keeping quality, etc., must be determined. At the present time, 2.8×10^6 rad** has been considered the most likely level of ionizing radiation which will be used commercially, allowing a safety tolerance above the 2×10^6 rep level established for sterilization. But since there is no way of knowing how an irradiated product will be used at some future date, deliberate exaggeration of dosage was suggested, also, as a means of acquiring information necessary for future use.

Under the plan suggested, irradiation studies must determine nutritional adequacy and possible toxicity. Since this is difficult to do in the same experimental situation, nutritional adequacy has received more attention, with primary emphasis upon longevity, reproduction, and growth. No significant adverse effects have been found in these gross measurements, but odor and flavor changes in irradiated foods have been widely noted.

Maleby ('58) in using cathode ray-irradiated wheat in dosages from 5×10^4 to 1×10^6 rep found the diets nutritionally adequate for growth and the protein readily available to the rat at these levels. Cannon ('59) using both cathode ray- and gamma-irradiated wheat varying

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**rad is equivalent to the absorption of 100 ergs per unit gram of density. One rad unit is equal to 93/100 rep.

in dosage from 0.28×10^6 to 9.3×10^6 rad has suggested that neither the type of radiation nor the level of irradiation affects the value of wheat protein except at the very highest gamma level, and that the effect of the two types of radiation appears to be slightly different.

Cannon ('59) maintained rats for ten weeks on the various irradiated diets. In addition to growth measurements, protein quality was assessed using the enzyme system, liver xanthine oxidase. Per cent of fat was determined in the livers, also. An interesting trend was noted in animals consuming the cathode ray diets which was not observed in the gamma diets. As the level of irradiation increased, there was an increase in liver fat accompanied by a small decrease in xanthine oxidase activity. Carroll ('60) working with threonine deficient casein diets has shown this relationship to be a significant adaptation mechanism. Possibly the altered fat levels found in the rat livers as part of Cannon's ('59) study may point to a more specific undesirable side-effect of irradiated feedings.

Werhes and Lehman ('56) discussing the food additives situation declared that for all practical purposes, ionizing radiation is a food additive and that the fate of this ingested food additive must be understood in normal as well as in subclinical physical conditions. The safety problem is most complex and difficult because the flavor and odor changes point to chemical reactions within the food which in turn suggest nutrient changes with new and possibly toxic end-products. The authors pointed out that:

> Sole reliance on findings of orthodox toxicological study of irradiated foods themselves, in ignorance of both the identity and quantity of substances therein that may influence such findings, invites valid reservations to any final conclusions.

The desirable advantages of irradiation preservation of foods necessitates continued research in this field. If the high levels of irradiation do produce changes in the chemical compounds of foods so preserved which would be toxic to animals, then these must be carefully studied. And in turn, animal findings must be evaluated with reference to the heterogenous nature of man and every assurance given to man that irradiated preservation of foods will be beneficial to him.

This study was undertaken in an attempt to further evaluate the effects of feeding cathode ray- and gamma-irradiated wheat protein to experimental animals. It was designed as a nutritional study and no attempt has been made to determine chemical changes in the wheat. In addition to growth of the rat, the more sensitive techniques of enzyme system and histological evaluations of liver tissue have been used.

REVIEW OF LITERATURE

Ionising Radiation

Ionisation is a molecular change induced by an energy source that increases the chemical reactivity within the molecule. There are certain types of radiation energy which may blaze a trail through a material. There are those which are active agents themselves colliding with molecules, and there are those that can cause the ejection of electrons from atoms of the material. As a result of this process the remainder of the atom acquires a positive charge, and a pair of "ions" has been formed out of an electrically neutral atom. This may be called an "ion-pair" formation, and is a fundamental process which must be understood in considering the biological effects of any radiation. When a molecule is ionized, it generally breaks up but since the pieces are unable to escape, they react and re-react until the most stable combination can be found. (Ellinger, '57). The ionizing source used, the mechanism of its effect, and the application it finds may be considered somewhat new but the appearance of new and largely unknown chemical substances in a product are not. This is an old problem encountered with all methods of food preservation.

There are two basic problems to consider when choosing the energy source of ionization for food preservation. One of these is the source itself, whether machine or isotope, and the other is the type of radiation which must be such that no nuclear transformations will occur. The sources of irradiation for food preservation are chiefly electron

accelerating machines and radioactive isotopes; the possible types of radiation are x-rays, gamma waves, cathode rays, and beta rays.

The electron accelerating machines in common use are basically of four types: resonant transformers, Van de Graaff, linear, and the Capacitron. All of them operate on the principle of projecting artificially produced electrons (commonly designated cathode rays) directly at a material as it passes through the field. (Evans, '55). The electron accelerating sources are advantageous because they may be stopped or started at will, do not require excessive precautionary measures, and are available at outputs sufficient for large scale processing.

The isotopic sources may be of several kinds. There are gamma-emitting radioactive isotopes such as Cobalt-60 which may be produced in atomic reactors. There are fission isotopes such as Cesium-137 (gamma-emitting) which may be separated from the combinations of radioactive elements remaining after the unused uranium is removed from spent reactor fuel. There are the completely spent or nearly completely spent fuel elements available from reactors and stored as waste products until they can be returned to processing plants for recovery of fissionable material. Probably the greatest advantage of isotopic sources is that they will constantly emit at known rates and energies but since they are constantly emitting, extreme precautionary measures must be used to protect personnel. (Brownell and co-authors, '57).

The two most common forms of radiant energy considered suitable for bombarding food material in food preservation are cathode rays produced by machine sources, and gamma waves emitted from isotopic sources. The gamma waves are found in the upper frequencies of the

electromagnetic spectrum and consist of electric and magnetic vibrations which travel in straight lines through a material but perpendicular to one another. The terms cathode ray and beta-irradiation are used interchangably because they are physically identical although true beta particles are emitted only by radioisotopes.

The ability of these two sources of radiation to penetrate and therefore produce ionization throughout a material varies. Goldblith and Proctor ('54) claim the cathode ray distribution of energy within a material is non-uniform and that with a 0.55 gm per cm^2 thickness of material, the variation in dosage received from the top to the bottom of the material may be as much as 40 per cent. Therefore, it is highly important in using this source that this lack of uniformity be minimized as much as possible; for instance, with wheat, only one layer thickness of grain kernel is passed under the electron generating beam at a time. The gamma emitting sources, however, penetrate deeply into a material giving a more uniform effect. This source itself has high activity and its location in relation to the material absorbing is important.

The energy introduced via cathode ray- and gamma-irradiation is thought to ionize a given material either directly or indirectly or through a combination of both. The biological action of irradiation, regardless of the approach, may have a four-fold effect: a primary physical process, a secondary physical process, a primary biological process, and a secondary biological process. The direct approach or the Target theory as it is called suggests that energy is released directly in the molecular structure at the most sensitive area with the primary physical reaction and the primary biological reaction taking

place at the same location within the material or cell. The extent of damage will depend upon the degree of radiosensitivity within the material, with embryonic and immature cells undergoing the most extensive changes. Bacteria, materials with low moisture content, and fats are thought to be affected in this way. (Ellinger, '57).

Alexander and co-authors ('60) have recently postulated that the primary physical process is the biologically important stage in direct ionization. After subjecting crystalline serum bovine albumin to energies obtained from a Van de Graaff generator, and subsequently studying solubility, hydrolysis, and ultracentrifugation results, the authors concluded that the first effect of a direct "hit" in the protein was to alter the shape of the molecule. Then with a second "hit," the molecule appeared to open out still further until one-half of the disulfide bonds were accessible. And finally, with a third "hit," the chemical changes in the molecule became significant with losses of carboxyl, amino, and disulfide groups.

The indirect theory proposes that irradiation creates free radicals in the body of the material which diffuse to the surface of the nearest molecule and then react. The primary physical process may take place in one area, then the energy diffusion of either a physical or chemical nature brings about the biological changes in another area. The production of these free radicals in contrast to organic radicals depends upon an aqueous medium, which is readily available in food materials. The primary physical reaction has been described by O'Meara ('52) as follows:

$$H_2O \longrightarrow H_2O^{+} + e^{-}$$

$$H_2O^{+} \Rightarrow H^{+} + OH^{-}$$

$$H_2O + e^{-} \longrightarrow H + OH^{-}$$

These short-lived decomposition products of water initiate the production of long-lived radicals and peroxide-like derivatives which in turn effect oxidations and reductions throughout the material, particularly among the proteins and amino acids which are so closely associated with water molecules. Barron ('54) has suggested that the chief oxidizing radicals produced by ionizing radiations are OH, HO_2, and H_2O_2, the H_2O_2 having a negligible role, and the chief reducing radical is H^{+}. The total effect will vary with the quantity of various radicals present, their active life, and the presence in the solution of others competing for them. If the material has a high moisture content, it is more apt to undergo irradiation damage, and the over-all chemical effects have been found to be reduced when material is irradiated in the frozen state. (O'Meara, '52).

X-rays, gamma waves, and cathode rays are commonly measured in roentgen units, named for Konrad Roentgen who discovered in 1895 the first of the artificially produced radiations, X-rays. The International Commission Recommendations for Radiological units (1954) defined the roentgen as the unit or dose of radiation "such that the associated corpuscular emission per 0.001293 grams of air produces, in air, ions carrying one electrostatic unit of quantity of electricity of either one." In ionization terms, one roentgen produces about two ionizations per cubic micron of tissue. The "absorbing dose of any ionizing radiation is the amount of energy imparted to matter by the ionizing particles

per unit mass of irradiated material at the place of interest," and in the older literature, the term "rep" (roentgen equivalent physical) was used, usually refering to the unit of energy absorption equal to 93 ergs per gram of tissue. (Swanson, '57). The official term in use now is "rad," and one rad is equivalent to the absorption of 100 ergs per gram of tissue, so one "rad" unit is equivalent to 0.93 rep.

Radiation Effects Upon Protein and Amino Acid Composition

It is generally agreed that the irradiation effect is much greater in individual, isolated compounds than it is when the amino acids, sugars, and fatty acids are within the framework of a whole food. The density of the material may be great enough to slow down or minimize the ionising action. This protection which is afforded by intact food materials, may be of value in food preservation but individual results suggest that subtle irradiation effects can lead to deamination, decarboxylation, and polymerization of proteins, carbohydrates, and fats, any one of which could be responsible for damage to nutritive value and palatability.

Barron and co-workers ('55) treated simple amino acids with low levels of X-irradiation and suggested that the initial reaction was one of oxidative deamination. The authors postulated the following reactions in the presence of the oxidizing radicals, OH and HO_2:

$$CH_2NH_2COOH + OH \longrightarrow CHNH_2COOH + HO_2\cdot$$

$$CHNH_2COOH + HO_2 \rightarrow HC{=}NHCOOH + H_2O_2$$

Then if metal ions were present, H_2O_2 could react with amino acids, eventually giving CO_2, NH_3, aldehydes or acids with one less carbon atom.

Ammonia formation was found to be highest in acid and alkaline solutions indicating that pH influences the irradiation effect.

Barron ('54) and Dale and Davies ('51) have both shown that thiols and sulfur-containing amino acids are chemically altered when irradiated in dilute aqueous solutions. Ambe and co-workers ('60) in irradiating the amino acids cysteine, cystine, and methionine, glutathione, and other sulfyhydryl proteins found only traces of H_2S. Grady and co-authors ('55) have suggested that the reduction of sulfur amino acids probably prevents and diminishes irradiation damage by supplying electrons to damaged protein molecules in a material.

Dale ('40, '42) in working with enzyme protein, carboxypeptidase and d-amino acid oxidase, found that the more dilute the enzyme concentration, the more inactivation X-irradiation could produce. Glucose, fructose, and nucleic acids were found to be effective protective agents. The protein portion of the enzyme was more sensitive to irradiation than the phosthetic group. Loken and co-workers ('59) observed that albumin, cysteamine, cysteine, glucose or sucrose would protect the enzyme pepsin if one of them was in solution during irradiation.

Food materials have been used to study the effect of irradiation on amino acids. Proctor and Bhatia ('50) irradiated haddock fillets with 9×10^5, 2.7×10^6, 5.7×10^6 rep levels of cathode rays and observed no significant destruction of arginine, valine, histidine, leucine, lysine, methionine, phenylalanine, tryptophane, threonine, or cystine. Lysine, methionine, and tryptophane exhibited at the most only 9.8 per cent loss.

Tsein and Johnson ('59a) prepared protein hydrolysates of non-irradiated and irradiated beef and milk and studied the changes in amino acid composition after 2.8×10^6, 5.6×10^6, and 9.3×10^6 rad gamma-irradiation. Both products were frozen prior to irradiation since this was considered a desirable way to minimize the effect of free radical formation in water. Stein-Moore column chromatographic technique was used for amino acid determinations. At the 2.8×10^6 rad level, the following amino acids were found significantly reduced in milk: aspartic acid, serine, methionine, and isoleucine. At the 9.3×10^6 rad level; glutamic acid, glycine, leucine, phenylalanine, and histidine were found significantly reduced also. In the beef, the following order of amino acids were seriously reduced: glutamic acid, serine, aspartic acid, threonine, glycine, lysine, methionine, arginine, histidine, and proline. And in both the beef and the milk, the greatest reduction was found in glutamic acid, aspartic acid, serine, and glycine.

Radiation Effects on Nutritive Value of Foods

Various studies of irradiation effects in food materials have so far indicated no traces of radioactivity produced in the food. Meinke ('54) tested for radioactivity in 24 elemental food constituents ordinarily found in meat and found no detectable radioactivity following low levels of irradiation. However, much higher energy levels of irradiation need to be tested further before the possibility of induced radioactivity is completely discounted. The threshold energy level for the production of induced radioactivity is thought to be about ten million electron volts. (Hannah, '56).

Irradiation studies have for the most part been directed toward the nutritive value and possible toxicity in foods. Richardson and Brock ('58) reported, that in general, the data obtained in an 80-week feeding study with rats indicated the nutritive value of an irradiated diet was slightly less than that of a non-irradiated diet but the differences were so small as to be of questionable significance. Four generations of rats were followed and fed a synthetic diet based on soybean protein irradiated with 2.79×10^6 rad gamma. Life spans of both the control-fed and irradiation diet-fed animals were essentially the same; of the 80 females of each group who reproduced, the irradiated diet group produced 3467 young and weaned 84.7 per cent of the 2993 allowed to remain with them, while the control group produced 3290 young and weaned 90.0 per cent of the 2923 allowed to remain with them.

Fats and water are thought to be important targets for radiation damage in food materials. Since pork is a high lipid and high moisture content food material, it has been considered an excellent material for studying possible radiation damage. Bubl and Butts ('60) fed gamma-irradiated pork, which had been stored at room temperature for three to eight months prior to diet mixing, to rats through four generations. The pork was incorporated as 35 per cent of the dry weight of the diet. No statistically significant differences were obtained in growth, breeding, and longevity in the four generations.

Read and Kraybill ('58a) selected fourteen food items representative of different chemical systems and fed them ad libitum to rats for eight to twelve weeks. The foods were stored frozen following gamma-irradiation at 3×10^6 rep and 6×10^6 rep, prior to incorporation into

diets as 35 per cent dry weight. The following foods were investigated: ground beef, fresh ham, haddock fillets, boned turkey, sliced bacon, whole kernel corn, leaf spinach, sliced peeled beets, green snap beans, sliced peaches, whole strawberries, canned bread, military cereal bars, and whole powdered milk. Decreased growth was found in animals fed diets containing 6×10^6 rep irradiated sliced peaches, which may indicate that repeated ingestion of low level radiation by-products might establish a "toxic condition characterized by depressed growth." Significant weight gains were found among the rats fed irradiated cereal bar and corn, and this was attributed to the breakdown of cellular walls making it possible for the animals to utilize the foods better.

Very little work has been done in investigating the nutritive value of wheat subjected to irradiation. Since irradiation of wheat suggests improved insect infestation control, a study directed toward uncovering the effect of irradiation upon the nutritive value of wheat can be considered practical and desirable.

Wheat Protein

Wheat is a basic farm crop in the United States and a staple in the diet, and it affords an excellent material for irradiation studies. It combines the advantages of studying direct and indirect effects of irradiation upon the embryo and storage reserves as well as continued study of seedling growth and second generation properties. The unique constitution of its protein deems that it be understood before such use is made, however. Since the beginning work of Osborne and Mendel, wheat has been known as an inadequate protein, not biologically complete in its amino acid content.

Osborne and Mendel ('12), characterizing the gliadin portion of wheat protein, fed carefully extracted gliadin which has been dried to a fine powder to rats for varying periods of time. The gliadin was incorporated as 18 per cent of the diet with starch, sucrose, agar, salt mixture, and lard making up the balance. Long term feeding trials with young and old animals were done, and the older animals varied in their ability to remain on the diet without undue loss of weight from 79 to 290 days. The young animals were found to remain more or less at status quo, an indication that the gliadin was able to maintain the animal but not able to promote growth.

Gliadin at that time was generally considered to be different from animal proteins because it contained none or else a very small amount of the amino acid lysine. Since growth response was so prompt when even a limited amount of animal protein (which was thought to be high in lysine) was added to a gliadin diet, Osborne and Mendel ('14) undertook additional feeding trials using lysine supplements. An 18 per cent level of gliadin was again used in the basal diet and reduced only enough to add 0.54 per cent lysine in the supplemented diet. The animals were found to respond promptly to the addition of lysine by growing rapidly. When the lysine supplement was removed, growth stopped and did not begin again until resupplementation was started. The authors felt this gave conclusive evidence that lysine was indispensable for growth in the rat and apparently the rat was not able to synthesize lysine.

With the improvement in methods of determining exact quantities of amino acids present in proteins throughout the years, the F.A.O.

Nutritional Studies No. 16 ('57) established a "reference protein" as a guide for supplementing basal wheat diets. This "reference protein" established that the amino acids which will require supplementation in order to bring a basal wheat diet to a complete protein basis will be lysine, tryptophane, methionine, isoleucine, valine, and threonine. Lysine, the most limiting amino acid, has received attention, since it can not undergo reversible deamination nor can the carbon skeleton of the d-amino acid be used for the l-form synthesis, and all animals studied need lysine as a component part of the foods being taken into the body.

Lawrence and co-workers ('58) carried out microbiological assays for lysine on 286 samples of wheat, representing durum, triticum, and hybrids. The various varieties had a minimum of 2.46 per cent lysine to a maximum of 3.84 per cent per unit of protein, and the over-all average was 2.89 per cent for wheat of about 13.5 per cent or more protein. The authors reported no signficant differences in lysine content among the spring wheats, red spring wheats, and winter wheats, and no alterations due to year of growth or location except wherein the total protein level was affected. Below the protein level of 13.5 per cent, however, an inverse relationship in lysine content was found. It appeared that more lysine was available in the bran and germ and there was also a higher lysine content present in the endosperm in low-protein wheat. Perue and co-workers ('50) also showed the gluten-lysine content of 17 different flours derived from wheats of all the major types ranging from 5.7 to 14.2 per cent nitrogen to be consistently higher in the low-protein flour samples.

Animals fed with a diet containing small amount of lysine may not show outstanding symptoms of a deficiency, and Harris and co-workers ('43) have suggested that for the young rat, lysine may be required purely as a "passive material" for synthesis of proteins. Using an 18 per cent level of corn or wheat and starting with three week old rats, rats were fed for five, six, and seven weeks on supplemented and non-supplemented diets. Those diets with the smallest amounts of lysine (controls) resulted in cessation of growth as Osborne and Mendel had shown and hypoproteinaemia as well. It was felt that the blood picture obtained indicated not so much an anemia as a retarded development of the haematopoietic system. Radiological examinations showed decreased subcutaneous fat, waste of muscle, and reduction of bone calcification. Apparently the animal body in detecting the absence of lysine reduced growth of some organs and transfered the available amino acids to organs with higher growth priorities so that status quo could be maintained.

Since wheat is so important in the economy and diet of the people of the United States and the world, any process applied to it which may affect the limiting amino acid lysine needs further investigation. Barcroft ('39) in arranging foods into three groups based upon the time they could be preserved satisfactorily in storage for emergency purposes classified wheat among those that may be kept for ten years or longer. When it is considered that irradiation would greatly increase this projected estimate, any nutritive value study utilizing irradiated wheat must attempt to investigate any effect on lysine.

Irradiated Wheat Protein

Irradiation in low doses does appear to affect the physical and chemical properties of wheat in ways which alter its storage and baking qualities. Grains are commonly thought to be more affected by direct ionization than by indirect since better quality wheat tends to have a low moisture content.

Conger and Randolph ('59) observed wheat seed embryos, before, and after irradiation and storage for free radical formation. Ground wheat germ was used because the majority of the primary nutrients of the seed are located in the embryo and biological damage is most apt to occur there.

Both x-rays and Cobalt-60 gamma rays were used, varying from 1×10^5 to 6×10^5 roentgen units, and measurements were made from three minutes to 30 days following irradiation. The moisture content was carefully controlled by limiting the dry germ to 1.5 per cent by weight and the wet germ to 8.5 per cent by weight. One hundred milligram samples were used to detect and measure electron resonances with each resonance presumed to represent a free radical. The non-irradiated wheat embryos whether wet or dry gave no detectable yield of free organic radicals but the irradiated wheat germ gave a broad radical signal, very similar to those obtained from other irradiated proteins (Grody and co-workers, '55). Two to three times as many radicals were detected in the dry wheat germ after irradiation as were found in the wet, approximately 10^{11} radicals per milligram of dry weight of germ irradiated dry in air per one-kilo-roentgen of Cobalt-60. Similar results were obtained whether the irradiation took place in air, or in

an atmosphere of oxygen or nitrogen. The authors crudely fractionated the wheat germ into component parts in an effort to determine in which area the largest share of radicals resided. The fats and oils gave no detectable signal even after doses 100 times as large as that required for whole germ. The carbohydrate fraction gave a slightly different signal, as yet unexplained, but for equal dry weights and doses the water-soluble protein-nucleic acid fraction gave a signal averaging 2.8 times as great as the dry whole germ.

Although the post-irradiation radicals were not identified, it was found that radicals decayed rapidly within the first ten minutes and then progressively less rapidly as storage time increased. It was suggested by the authors that the wide difference in radical quantity noted between the dry and wet wheat germ might be due to the more rapid decay of the radicals noted in the wet germ and that biological differences noted later in the seeds may be due to this decay rate.

Yen, Milner, and Ward ('56) in addition to storage studies found after using gamma radiation at zero, 2.5×10^4, 6.25×10^5, 1.875×10^6, and 3.75×10^6 rep levels that germination could be eliminated at 1.25×10^5 rep if the moisture was maintained at 20 per cent but 6.25×10^5 rep was necessary for eliminating germination in a 12 per cent moisture wheat. Fat-splitting did not occur with dosages as high as 3.75×10^6 rep but there was marked increase in fluorescence of the grain extracts after irradiation at high levels. This was an indication of the interaction of carbohydrate and protein and suggests germ damage. The two highest dosages produced a small but definite loss in protein solubility as measured by turbidity of saline extracts and this was found

strongly intensified by storage, much greater than was found in the non-irradiated wheat storage. In those instances in which the wheat was wetted prior to irradiation, the wet grain was apparently less resistant to all the above changes.

Milner and Yen ('56) also studied the baking qualities of wheat gamma-irradiated at zero, 1.25×10^4, 2.5×10^4, 5×10^5, and 1×10^6 rep levels. In investigating the wheat prior to milling, decreased germination but no immediate changes in protein solubility and fluorescence at these lower levels was found. But the flour milled from the same wheat showed a decreasing ability to hold water as irradiation levels increased, and drastic reduction in viscosity. These findings agree with work reported by Brownell and co-authors ('55) in which bread flour was found to be more starch-like, drier, and less able to bind moisture when subjected to dosages of gamma-irradiation above 5×10^4 rep.

Alsup ('59) reported definite changes in the protein structure at dosages above 3×10^6 rep when crude gluten was extracted from irradiated flours. Up to 1×10^6 rep, the weight of crude gluten was increased indicating increasing water retention but after the 3×10^6 rep level yields were very small. Electrophoretic analysis by the moving boundary method was attempted in order to study changes in the protein structure, and it was found that above 1×10^6 rep, the six separate protein component peaks noted in the non-irradiated wheat controls gradually changed, until only two main peaks were distinguishable at 1×10^7 rep. The total nitrogen levels remained the same so one could suspect that there must have been a change or alteration within the

structure of the protein itself. All of these investigators have reported off-flavors and palatability changes in the irradiated wheat products.

It is possible that the physical alterations in the wheat protein may be due to partial destruction of essential amino acids or the binding of them so they are no longer available to the animal. Tsien and Johnson ('59b) observed changes in the amino acid content of lima beans and garden peas due to irradiation. The authors used hydrolysates of gamma-irradiated and non-irradiated peas and beans and the Stein-Moore chromatographic technique. Amino acid assays showed marked destruction of the lysine and arginine with destruction increasing with increasing irradiation.

	Control	2.8×10^6 rad	9.3×10^6 rad
Lysine	$10.44 \pm .05$	$9.21 \pm .09$	$5.83 \pm .07$
Arginine	$7.57 \pm .01$	$6.05 \pm .08$	$4.23 \pm .06$

Further work by Metta and Johnson ('59) in studying the affect of gamma-irradiation and heat sterilization upon the nutritional value of corn protein and wheat gluten indicated no changes in the lysine content of the corn or the wheat gluten. A high protein corn and commercial wheat gluten were used, suspended in water, frozen, then irradiated, 2.79×10^6 rad gamma for wheat and 2.79 and 9.3×10^6 rad gamma for the corn. The samples were dried, ground, and proximate analysis redone prior to diet preparation, providing 10 per cent protein, for rat growth studies. The animals on irradiated wheat diets averaged nine grams of food intake per day, growing at a rate of 0.55 grams per day. Proximate analysis showed that neither the lysine

content of the wheat or the corn was affected by heat processing or by the level of irradiation used, and the animals accepted the diets readily.

In outlining the general course to pursue in establishing the feasibility of ionizing radiation for food preservation, Lehman and Lang ('54) suggested that the amount of research would have to be somewhat proportional to the scope of application in preservation. If there are purposes for which irradiation preservation is better suited than any other method of preservation, then more risk can be justifiably accepted. Heat processing has been criticized for its alteration of physical and chemical properties of food and if irradiation sterilization could alleviate this, be proven nutritionally adequate, and non-toxic, it could become a valuable preservation method.

METHODS OF EVALUATING RADIATION DAMAGE TO NUTRITIVE VALUE OF FOOD

Protein Quality

The primary emphasis in studies of the nutritional adequacy of irradiated food products has been directed toward reproduction, longevity, and growth. Any food product when fed to an animal must provide to the cells of the animal body protein adequate in amino acid content so that tissue may be built, maintained, and repaired. Two methods of assaying protein quality, nitrogen balance and protein efficiency ratio, have been used.

Nitrogen balance, determining the nitrogen in the food consumed and the nitrogen of the excreta under controlled conditions, is a quantitative measure of the gain or loss of protein from the animal body. If the animal body is found to be in positive nitrogen balance or in a state of having sufficient protein for maintenance, repair, and growth, a more exact measure of the effect of protein quality upon growth is possible than is provided by simple weight increase (Maynard and Loosli, '56).

Maleby ('58), feeding rats diets composed of 14 per cent wheat protein and irradiated with cathode ray at 5×10^4, 1×10^5, 5×10^5, and 1×10^6 rep levels, determined nitrogen balance. All the diets were equally acceptable to the animals; all animals were found to be in positive nitrogen balance, and no significance was found among the diets in utilizing this measure of protein quality. Cannon ('59) also

determine nitrogen balance of animals maintained on cathode ray- and gamma-irradiated wheat diets varying in dosage from 0.28×10^6 to 9.3×10^6 rad. Only in results obtained from feeding the gamma-irradiated wheat diet of 9.3×10^6 rad was any decrease in nitrogen balance observed and found statistically significant. Apparently neither cathode ray- nor gamma-irradiation up to the level of 2.8×10^6 rad affects the nutritive value of wheat protein when measured in this way.

Another measurement of protein quality is given by measuring protein efficiency ratios. Protein efficiency ratios in terms of gain in body weight per gram of protein or nitrogen fed express the growth-promoting ability of a protein, and the greater the ratio, the better the animal cell is able to utilize the protein provided for necessary body functions. However, this measurement has its limitations since the protein content of the actual gain in body weight may be variable.

Protein efficiency ratio received its initial usefulness from work of Osborne and Mendel who felt that prolonged growth was a good criterion for protein synthesis. In 1915, these authors reported attempts to establish the absolute protein intake at which exact maintenance of body weight could be found, the intake at which slight decline could be found, and the intake at which slight gain could be found. Gliadin was among the proteins used in the study but the results obtained were so variable that high value could not be placed upon them. However, they did show that when gliadin was the source of protein in the rat diet there was a slight decline in weight on a 9 per cent protein level and a slight increase in weight with a 10.5 per cent protein level, ad libitum feeding. It was noted that in young

animals the protein intake had to be decreased beyond the point where growth just ceased since there appeared to be a considerable range below this at which maintenance was still possible. The maximum efficiency of the different proteins was found to vary with the type of protein supplied by the diet.

Barnes and co-authors ('45) in investigating the various methods of studying protein quality suggested that the efficiencies of animal proteins are highest when fed at low levels such as 8 to 12 per cent, but that efficiencies of cereal proteins improve as the level approaches 20 per cent of the diet. On the basis of protein efficiency, it would appear that a dietary level of protein which can give an efficiency ratio of 2.0 or greater would need to supply only 10 per cent of the diet. The authors suggested that no matter what experimental approach was used, paired-feeding or ad libitum feeding, if the protein level of the diet was adjusted so that about 0.9 grams of protein could be retained each day, then the efficiency of the protein would be close to its maximum.

Recent research reported by Campbell ('60) has attempted to clarify some of the variables associated with protein efficiency ratio determinations. Using a basic diet furnishing 10 per cent protein and 10 per cent fat and correcting back to a casein reference standard, the author found that marked differences in protein efficiency ratios result from differences in age of the animals. The protein efficiency ratio derived from casein decreased as the age and time on the test increased. When casein was compared with plant protein sources, the differences noted in protein efficiency ratios were greatest during the early part

of a four week experimental period. When a 10 per cent level of either protein source was fed, the protein efficiency ratios were near maximum values. The author concluded that if only one level of protein could be used in an experimental situation, then the 10 per cent level of protein would be satisfactory regardless of the type of protein, if all variables were carefully controlled.

Before irradiated foods can be commercially feasible for human consumption, work must be done at the micro level also. It is known that only a small percentage of the compounds in a food are immediately affected by irradiation (Goldblith and Proctor, '55). But often a tiny amount of any product or chemical reaction can bring about extensive biological and/or physiological changes. The final determination of suitability will have to rest with a large body of biological data more extensive than afforded by growth and protein efficiency studies.

Enzymes and Liver Fat

Lehman and Lang ('54) suggested that enzyme systems which are such important units of animal metabolism should be studied as a means of exploring every possible facet of the metabolism of irradiated foods and their possible damage to the animal body. One of the most sensitive enzyme systems for indicating quality of protein being fed into a system is liver xanthine oxidase.

Litwack and co-authors ('52; '53a) in studies of the liver xanthine oxidase activity of rats fed casein, whole gliadin, and gliadin supplemented with various amino acids found that this enzyme system is especially sensitive to the amino acid availability in the dietary

proteins. Any measurement of its activity actually measures the formation of that enzyme protein, and this has been found to correlate well with growth results without the added interference of hormonal state, water intake, and balance, etc. When the gliadin was supplemented with tryptophane to bring it to the level present in casein, greater activity was noted than with whole gliadin alone. When lysine and tryptophane supplements were added to gliadin, a still greater response was found indicating that the lysine was the most important factor lacking in the gliadin to stimulate the processes of growth as well as enzyme formation.

Bothwell and Williams ('54) studied the effects of a lysine-free ration upon the liver xanthine oxidase activity in weanling rats fed either forcibly or ad libitum. The rats were not started on the purified amino acid ration until between 55 and 60 grams in weight and then maintained for ten to fourteen days. Rats force-fed the lysine deficient ration began to die earlier but no matter how the ration was fed, the lysine deficient diet resulted in a small but definite reduction in liver nitrogen content and reduction of the liver xanthine oxidase activity to somewhat over one-half that of the controls. The lysine-free ration was found to give a completely different enzyme picture in comparison to a histidine-free ration which had no effect and a methionine-free ration which completely depressed enzyme activity.

Where living tissues are concerned, radiant energy invariably produces injurious effects. Besides the mutations which may occur, mitotic activity may be depressed, the synthesis of DNA may be slowed down, clumping and fragmentation of chromosomes may occur, as well as

various cellular changes such as vacuolization, increased fatty changes, and cell death (Swanson, '57). The action of ionizing radiation is thought to be oxidative in nature and possibly directed at the oxidative activities of the cell. A study of the enzyme systems directly involved in the electron transport could possible shed additional light on the adequacy of irradiated foods.

Benditt and co-workers ('49) fed a non-irradiated protein-deficient diet to rats and used cytochrome oxidase activity as an indication of protein quality. The authors found that the cytochrome oxidase activity decreased progressively with time and somewhat faster than the liver protein decreased. Cytochrome oxidase would provide a means of studying a mitochondrial enzyme system of the cell while liver xanthine oxidase would provide an excellent means of studying a soluble cytoplasmic enzyme system.

Read and co-workers ('58b) are the only investigators who have included evaluations of both enzyme systems in irradiated feed studies. The authors have reported that no changes were found in the xanthine oxidase system but that the cytochrome oxidase system increased in activity throughout a four generation study of rats. The diets used were composites of nine frozen-stored 6×10^6 rep gamma items: beef, pork, bacon, haddock, greenbeans, beets, peaches, powdered milk, and military cereal bar.

Work has been done in the Foods and Nutrition Department, Michigan State University, concerning the ability of an animal system to adapt to or adjust itself to the dietary protein fed. Carroll ('60)

working with 9 per cent casein diets deficient in threonine found the liver xanthine oxidase system depressed to a maximum point in 19 days after which it began to recover. Along with this decrease, the per cent of liver fat increased, reaching its highest point five days after the xanthine oxidase maximum depression. Then the fat began to move out of the liver, and the fat returned to its initial level following the lead of the recovering xanthine oxidase system. In addition, to the xanthine oxidase system, cytochrome oxidase was studied. Cytochrome oxidase, however, was found to be depressed throughout the experimental periods and did not tend to recover. Since cytochrome oxidase is so intimately associated with the oxidative processes of the cells, any depression here would have wide effect in all the cell's activities.

Cannon ('59) in studying the nutritive value of cathode ray- and gamma-irradiated wheat from zero to 9.3×10^6 rad levels found that as the per cent of liver fat on a dry weight basis increased the liver xanthine oxidase activity decreased. The progressive increase noted in the liver fat levels with increasing radiation levels was significant. This could suggest a possible alteration in the irradiated wheat which the animal was capable of detecting. The liver nitrogen levels in this work and that of Carroll ('60) showed no significant increase or decrease with the various diets used.

Since the animal liver is vital to the well-being of the animal, rapid changes in its size and composition may be due to the protein and/or glycogen being quickly added or withdrawn. And either of these will carry with it a multiple of its weight in water. The amount of protein present in the average liver cell may vary with the availability,

quantity of, and the amino acid composition of the dietary protein. As the animal body adjusts to the dietary intake, fat may be called upon to move also. In starvation, for instance, as the liver is depleted of its proteins, fat will move quickly into the liver cells until the depots are empty.

Fat infiltration of the liver under numerous conditions may be thought of as an alteration in the normal processes governing the fat content of the liver. An excess of fat may be due to increased formation of fat, decreased oxidation of fat, increased flow of fat from the depots, and impaired removal from the liver. And any one of these may be the secondary result of a primary factor. These basic primary factors have been catalogued by Popper and Schaffner ('57).

1. Imbalance
    - A. Nutritional factors
        1. Starvation
        2. Low-protein diet: Methionine deficiency; cystine deficiency
        3. High-fat diet
        4. High carbohydrate diet
        5. Lipotropic deficiency
        6. Vitamin imbalance: thiamine excess; biotin excess.
    - B. Metabolic factors
        1. Pituitary hormones
        2. Adrenal cortical hormones
        3. Thyroid deficiency or excess
        4. Insulin deficiency

5. Sex hormones

6. Central nervous system influence

7. Obesity.

2. Toxic factors

A. Chemical poisons: carbon tetrachloride; chloroform; phosphorus; trinitrotoluene.

B. Bacterial toxins

C. Anoxic factors; anemia; congestion

3. Combined factors

A. Alcoholic fatty liver

In studying this complicated interaction of dietary protein-, liver-, and fat metabolism, growth periods may be of great help. During growth, the animal body is called upon to utilize everything at its command to develop the animal. If there is any deficiency apparent in the dietary protein, the general metabolic stimulation created by the growth process can aggrevate the deficiency. Histology is another research tool which may be used for studying the aggrevation at the cellular level.

Histology

There is such a wide variety of biological events which may be attributed to irradiation that no single response has been found to be unique for radiation damage (Patt, '53). Likewise, the feeding of irradiated foods and their subsequent metabolism in the animal cell might be suspected of as yet undetected effects. According to Ellinger ('57), there is disagreement on the effect irradiation has on liver tissue proper, probably due to the cyclic changes in cells following

irradiation and the enormous repair power of the liver. But cellular and functional changes do occur as well as reactions to toxic substances and any intimate examination of liver tissue such as histology affords could possibly indicate effects attributable to the feeding of irradiated foods.

The liver is one of the largest organs in the animal body, and it is one of the most homogenous of organs regarding its primary cell type. The classic lobule of the liver is established by the location of central veins and portal areas. The portal areas are composed of bile ducts, lymph channels, and blood vessels. They are the gateway for nutrients to the liver cells. The liver cells are thought to be no more than two cell thicknesses and are arranged in cords surrounded by continuous ducts called sinusoids. Most of the metabolic products of the cells are emptied into the sinusoids and gradually are pooled in the central vein areas from which they will find their way out of the liver and into the body as a whole. Using this concept of the liver lobule, there must be a large number of portal areas in comparison to the central vein areas. This gives the classic concept of the liver lobule a characteristic hexagon appearance. (Copenhaver and Johnson, '58).

The average liver cell of the rat may contain one or two nuclei which may have one, two, or four nucleoli in them. This can give the rat liver cell a high degree of DNA. Mitochondria are also prominent in the liver cell, and it is here that the oxidation-reduction activities of the cell are thought to be carried out as well as fat metabolism.

In the normal liver, fat is found in the hepatic cells and the Kupffer cells in the form of small droplets. This can be demonstrated by sensitive histological techniques. The rat liver, however, ordinarily does not show fat deposition by any of the routine methods. Popper and Schaffner ('57) have attempted to classify the various patterns of fat deposition which have become known through research studies, first, according to its position in the liver cell itself, and secondly, according to its lobular location.

Cellular classification:

1. Perisinusoidal pattern. Fine droplets, like beads on a string, lie at the edge of the liver cell wall next to the sinusoids. Under abnormal conditions they grow in size.

2. Perinuclear pattern. Fat droplets will be found surrounding the nucleus or located in the center of the hepatic cells.

3. Diffuse small droplet pattern. Fat droplets will be found surrounding the nucleus or located in the center of the hepatic cells.

4. Large droplet deposition. Small fat droplets will coalesce to form one or two large droplets which gradually become one huge droplet. It will replace the entire cytoplasm in time and push the nucleus to the side. As this fat droplet continues to grow, it will break the cellular membrane and two huge fat droplets will coalesce to form a fatty cyst, enabling greater fat storage.

5. Diffuse fat. Grossly the liver will be enlarged, yellow, and even doughy in consistency. Every cell will contain excess fat, readily shown chemically, and sinusoids will be narrow. Since this class is actually an exaggeration of the central and peripheral forms, it is usually nutritional, toxic, or endochrine in origin.

The classification on the lobular level is independent of the type of fat deposited within the cell itself except the perinuclear fat which is usually centrolobularly located.

1. Scattered fat. Isolated fat rich cells can be found throughout the normal liver lobule and will be seen in high carbohydrate diets.

2. Centrolobular fat. This occurs readily with choline deficiency. The fat rich cells will appear first and disappear last from the central vein area. The condition will be noted on gross examination because the liver will show definite yellow areas.

3. Intermediary fat. Whenever the central zone area can no longer function, the intermediary zone will take over. It will be active in severe passive congestion and carbon tetrachloride poisoning.

4. Peripheral fat. This will be found most commonly in toxemias and as result of other misfunctions such as altered protein metabolism.

Since the fat deposition of the liver can be correlated with various nutritional conditions and the type of protein being eaten, there is a wide body of histological literature which has accumulated. Much work has been done using a 9 per cent casein as the protein source of the diet studying the variance of individual amino acid levels and choline in relation to the fat picture. Nino-Herrera and co-workers ('54), varying the choline in the diets of male weanling rats on a 9 per cent casein diet, reported that histological findings could differentiate the choline deficient animals from the low protein plus choline diet animals. The choline deficient animals in this study showed diffuse fatty infiltration most severe in the central vein area. Whereas the low protein diet plus choline showed fatty cells distributed in zones interspersed among zones of normal cells with only rare fat cells being found in the central vein area. The 9 per cent casein plus choline animals had a per cent dry weight liver fat of 8.7 but the choline deficient animals averaged 27.3 per cent. It was noted that there existed a close correlation between the extent of a fat deposit as

determine chemically and the severity of the fat infiltration noted histologically.

Phenylalanine, leucine, histidine, tryptophane, methionine, as well as threonine and lysine have been individually studied. Adamstone and Spector ('50) fed casein hydrolysates to show tryptophane influence upon fat deposition. The animals were on the diet for three weeks and then killed at approximately seven, fourteen, and twenty-one day intervals. A heavy accumulation of fat was found in the liver. During the first week the fat was primarily peripheral and periportal with moderate sized droplets. The second week, larger droplets appeared, and within nine to fifteen days, an increasing amount of fat was found around the central vein area with eventual complete infiltration of the lobule. In addition, a change was noted in the nucleus as the fat became more centrally located. Instead of retaining two to four small nucleoli, a single large nucleolus appeared in the nucleus.

Dick and co-workers ('52) showed fatty liver development by feeding diets deficient in threonine or lysine. The rats were started on the respective diets at 27 to 28 days of age and fed for 28 to 63 days. Although the chemical fat levels determined were not high, a fatty change was noted on histological examination. The threonine deficient diet animal livers showed small to medium sized fat droplets which distended the cytoplasm. Wherever there were fat rich cells, they compressed the sinusoids although the nuclei were usually still centrally located in the cells. One large nucleolus was prominent in the nucleus rather than the usual two to four smaller ones. The majority of the livers showed fat around the central vein area. When lysine deficient diets were fed, the histological examination revealed a much slower develop-

ment of similar fatty movement into the cells, and the nuclear changes were less prominent.

Another report on the suboptimal intake of lysine and threonine reported by Singel and co-authors ('53) showed that no large excess of fat would develop on diets completely deficient in threonine or lysine. But if graded levels of each were fed, fatty livers did develop. It was felt that in order to overcome the requirements of growth, the diets must be adequate for at least 80 per cent of the optimal growth rate.

Plant protein diets have been studied in relation to their effect upon fat deposit in the liver. Shils and co-workers ('54) showed that a portal type of fatty liver can be rapidly and consistently produced in weanling rats by feeding diets in which protein source was corn, rice, wheat or cassava. In one week on the corn meal diet excess fat appeared in the portal region of the liver lobule and during the twelve weeks of the study, just kept piling up. Even with very high fat levels, the portal areas were most seriously involved. When an unenriched wheat flour diet was fed, adjusted to 7.8 per cent protein level, the same number of portal placements as diffuse placements was found. These diet animals were killed after 21 to 28 days on the diet and although the fat levels were not as high as others from a chemical standpoint, it was felt there was enough evidence to indicate that the fat would just keep piling up on this diet also.

In work reported by Vennart and co-authors ('58) the addition of tryptophane and lysine to a diet based solely on corn reversed the portal fatty livers observed. The animals on the supplemented diets

showed normal or near normal levels of fat when killed at 28 days, but the control group showed portal fat with small droplets visable.

These studies point to a readily discernable picture of fat within the cells and lobules of the liver. When choline is deficient in the diet, a central displacement of the fat in the lobule may be found. With low protein levels or diets based on low biological value proteins or with amino acid deficient diets, a portal type of fat can be observed in the lobule. Now how do these patterns change when the protein of the diet has been subjected to ionizing radiation.

There have been few studies involving the histological differentiation of fat in liver tissue derived from animals fed irradiated diets. The majority of the work has been directed toward the detection of malignant and benign conditions which are found in animals subjected to external radiation.

Poling and co-authors ('55) conducted a long term extensive study feeding albino rats a diet composed of raw ground beef irradiated with 2×10^6 rep cathode ray. The feeding continued through three generations with a total of 2685 animals. The meat was equivalent to about 45 per cent of the diet solids and results regarding growth, adult size, efficiency of food utilization, reproduction, hematology, survival, pathology, and neoplasms were reported. Most of the histological work was directed toward the presence of malignant and benign neoplasms of which the authors found equal distribution between the experimental and control groups. Histologically, most of the tissues examined were normal, roughly 79 per cent in both groups. Of the slight to quite severe histological deviations noted, there were 18 classes according to nature and location. The most frequent was

hemosiderosis of the spleen found in nearly all adult animals, both control and experimental, which is of obscure significance. The fifth most frequent occurence was fatty livers but here again there was equal distribution between control and experimental groups. Nearly all the histological abnormalities observed in both groups were those of heterogenous, spontaneous changes which one would expect with aging rats.

Growing chicks have also been used for irradiation studies. White leghorn chickens were raised and maintained for thirteen months on a wet-mash diet irradiated with 3×10^6 rep gamma. The proper Ralston-Purina Company diets for each growth level were used but complete vitamin mix was added to each one. Burns and co-workers ('56) reported the study and gross and histological findings were observed. The pathology of the birds showed no obvious abnormalities as being attributable to one group or another. Extensive fat infiltration in the livers of some of the animals fed irradiated diets was noted, however.

One other study, Ousterhout ('60) has been reported with rats maintained through three generations on a diet composed of 1.68×10^6 rep gamma-irradiated butter-fat, along with skim milk powder, ground whole wheat, salt, and vitamin A and D supplements. Extensive pathology was done but not reported in detail. All changes found were essentially the same in the control and experimental animals with equal frequency in both groups. No evidence of malignant tumors was found. Although histological work has been done using various plant protein sources, none have been published in which irradiated wheat was the primary dietary source.

Cannon ('59) suggested a possible adaptive mechanism on the part of the rat in the complicated picture of irradiated protein, liver, enzyme, and fat deposition. Although experimental fatty infiltration had not given any convincing indication of significant impairment of liver function, this does not rule out the possibility of damage yet undetected. It is known that the liver is more susceptible to injury when fatty, that detoxification of injurious substances may be faulty, and that the liver may be more sensitive to toxins.

If the picture previously reported was an adaptive mechanism, possibly the initial point of adaption could be detected with a shorter growth and feeding trial. If there were alterations in the irradiated protein molecule, possibly the animal liver cell could detect these enough to bring about changes in the enzyme systems. And if fat is being moved into the liver cells as a secondary result of protein alteration, histological sections could give indications as to its nature and manner.

This study was undertaken to determine the effect of 28 days of feeding cathode-ray- and gamma-irradiated wheat, at levels of 0.28 x 10^6, 0.93 x 10^6, 2.8 x 10^6, 9.3 x 10^6 rad, upon the liver xanthine oxidase and cytochrome oxidase systems, and the deposition of fat in the rat liver.

EXPERIMENTAL PROCEDURE

A 15 per cent protein wheat obtained from the Agricultural College of Michigan State University was used in this study. It was divided into nine lots, one of which was not exposed to radiation and served as a control for the entire study. Of the remaining eight lots, four were exposed to cathode ray-radiation from a high energy electron beam generator[1] and the other four were exposed to gamma-radiation emitted by Cobalt-60[2]. The four levels of irradiation used with each type were 0.28×10^6 rad, 0.93×10^6 rad, 2.8×10^6 rad, and 9.3×10^6 rad. Each of the nine samples of wheat was ground and incorporated into diets to provide 12 per cent protein. The composition of each diet was as follows:

TABLE I

Composition of diet

	%
Wheat	75
Corn oil	5
Mineral Mix*	4
Vitamin Mix**	2
Sucrose	14

* Wesson's Salts were obtained from the Nutritional Biochemicals Corporation of Cleveland, Ohio.

** The vitamin mix provided the following per kilogram of diet: 1 mgm of thiamin hydrochloride, 2.4 mgm of riboflavin, 1 mgm of pyridoxine hydrochloride, 3000 I.U. of vitamin D, and 298 I.U. of vitamin A.

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[1]Dept. of Agricultural Engineering, Michigan State University, East Lansing, Michigan.

[2]Phoenix Laboratory, University of Michigan, Ann Arbor, Michigan.

Male weanling albino rats weighing between 45 and 55 grams were allotted at random to the experimental diets so that each diet was fed to four rats. The same procedure was followed during a second experimental period two months later. A third group of four animals, a supplementary control for the histological part of the study, was fed a non-irradiated wheat diet whose protein content was 7 per cent.

The animals were placed at random into individual wire-bottomed cages and food and water were given ad libitum for a four week period. Records of weekly weight changes and daily records of food consumption were kept. On the 25th, 26th, 27th, and 28th days of each feeding period, one rat from each diet group was sacrificed by decapitation. The animals of each diet were sacrificed at varying times within the four days in order to minimize this source of bias as much as possible. The four animals of the low protein diet group were all sacrificed on the 27th day of that period.

After the animals were decapitated, livers were removed and xanthine oxidase and cytochrome oxidase activities were determined the same day. One lobe of each liver was placed in 10 per cent formaldehyde for later histological examination, and the remainder of the liver was frozen and stored for subsequent nitrogen and fat determinations.

Xanthine oxidase activity, expressed as micromoles of xanthine disappearing in one hour per unit weight of liver, was determined by the colorimetric assay of Litwack and co-workers ('53b). One modification (increased observation time) suggested by the procedure was utilized in this study in order to minimize the variability introduced by the immaturity of the animals. Although a long induction period

was noted in nearly all animals, first order reaction rates were eventually obtained. The cytochrome oxidase activity was determined spectrophotometrically as described by Smith ('55) using a reduced cytochrome oxidase solution and was expressed as a first order velocity constant in seconds^{-1} per unit weight of liver. Difficulty was found in keeping the cytochrome oxidase solution sufficiently reduced for determination of an entire day's samples. Maintaining the solution in a frozen state and re-reducing it mid-way through each day yielded somewhat more stability, however. During the reduction process, the nitrogen was bubbled through a solution of potassium pyrogallate as an additional precaution against oxidation.

The dried livers were analyzed for total nitrogen by the boric-acid modification of the Kjeldahl-Gunning method, (A.O.A.C., '57), fat was determined by ether extraction in the Goldfisch apparatus, and moisture was determined by difference after oven-drying. Since the liver weights were small in all the experimental groups and large amounts of wet weight were needed for the enzyme studies, it was necessary to do the ether extractions on extremely small amounts of dried liver. Therefore, it was deemed advisable to use a histological technique which could give an intimate picture of fat within the cell with regard to distribution and quantity. The liver lobe placed in 10 per cent formaldehyde at the time of sacrifice was allowed to fix until all the experimental groups were completed. Sections were prepared using the carbon dioxide freezing technique with some modifications. (See Appendix for method).

The data was evaluated by analysis of variance and by the T-test (Snedecor, '56) using the average of the irradiated diets as a unit against that of the control diet.

$$T = \frac{\text{average of the control - average of the experimental diets}}{\text{Standard deviation of the difference (derived from the remainder term of the analysis of variance)}}$$

Use of the T-test was considered because there was a consistent trend of depression noted in the xanthine oxidase and cytochrome oxidase measurements obtained from those animals fed the gamma-irradiated diets. The additional analysis was incorporated as a means of determining a possible significance of this trend. (See Appendix, Tables vii and viii).

RESULTS AND DISCUSSION

Osborneand Mendel ('15) pointed out that when the protein level of the diet is low an animal may try to eat more liberally in attempting to make-up the percentage deficiency of the ration by increased intake. The protein levels of all diets in this study varied from 11.8 per cent to 12.2 per cent which suggests little difference for practical considerations. The animals accepted the diets readily and at the time they were sacrificed had doubled their initial weight. No animals were lost during the study. The weather was extremely hot in the first experimental period, and although the animals were maintaied in an air conditioned laboratory throughout, additional stress was undoubtedly placed upon them by some variation in temperatures. Food intake records were kept on all animals during each experimental period. Food intake of animals receiving the irradiated diets tended to be greater than that of animals on the control wheat diets. (Table 2).

TABLE 2

Average food intake and weight gain for four week period

	Control		Cathode ray		Gamma	
Radiation dosage (rad)	Feed intake	Weight gain	Feed intake	Weight gain	Feed intake	Weight gain
None	191 gm	30 gm				
0.28×10^6			203 gm	34 gm	213 gm	38 gm
0.93×10^6			205	35	205	36
2.8×10^6			212	37	210	37
9.3×10^6			201	32	202	33
Average	191	30	205	34.5	208	36

There appeared to be no difference in the feed intakes and weight gains due to the type of radiation. However, there is some indication that the level of irradiation had some effect. The animals tended to grow at the same rate except for those on the highest level of irradiation who appeared to grow somewhat more slowly. The three lowest levels of irradiation measurements tended to be similar and greater than the control diet measurements. But the animals fed the highest level of irradiation, both cathode ray and gamma, showed weight gain averages more closely aligned with those found for animals on the control diets.

Cannon ('59) noted this same trend in the growth measurements of that study in which the animals were fed irradiated wheat diets containing 14.2 per cent protein and growth curves showed similar growth rates in animals fed diets made from wheat irradiated to the level of 2.8×10^6 rad. However, at the highest level of both cathode ray- and gamma-irradiation, 9.3×10^6 rad, a slower growth rate was noted. The author suggested that the animals on the highest level of irradiation might possibly have reached the same level of growth as the other animals if the study had not terminated at ten weeks. Since the same pattern of growth was found in this study when animals were under intensive growth stress, it may more logically suggest an alteration in the protein which the animal was not able to utilize as well. As Read and Kraybill ('58) suggested, it is possible that irradiation broke down the cellular walls of the wheat so that the animals found the wheat easier to eat and accept. However, at the higher level some other undetermined factors may have opposed this beneficial effect.

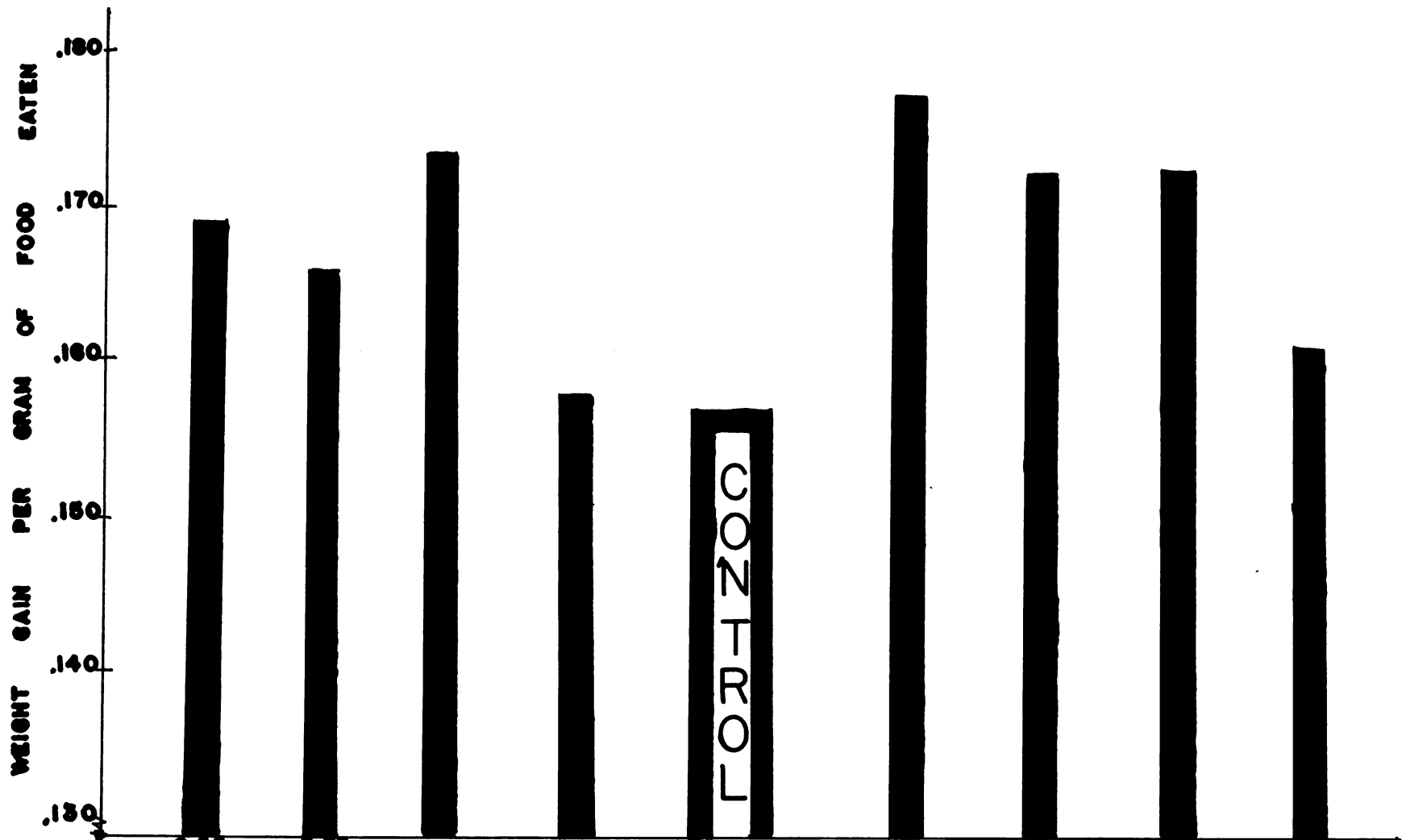


FIGURE 1: AVERAGE WEIGHT GAIN IN GRAMS PER GRAM OF FOOD EATEN

The average weight gains per gram of diet eaten (figure 1) were calculated also in order to determine whether the trend noted from the growth results would be altered when weight gains were corrected for feed intake. The trend persisted, appearing to be inversely related to the level of irradiation rather than to the type of radiation, as the animals fed the highest level of irradiation, both cathode ray and gamma, showed weight gain averages similar to those found for the animals on the control diets. The results were subjected to analysis of variance but were not found statistically significant. (See Appendix, Table iii).

Although weight gains and feed intakes do give a relative measure of growth and in turn indicate the protein quality of the feed, another measurement is that of protein efficiency ratio. These ratios in terms of gain in body weight per gram of protein or nitrogen fed, express the growth-promoting ability of a protein, and the greater the ratio, the better the animal cell is able to utilize the protein provided for necessary body functions. The protein efficiencies of the various irradiated diets used in this study were calculated and expressed as the weight gain per gram of protein eaten during the four week study. (Table 3). Very little difference was found in the average efficiency ratios obtained among the control and the irradiated diets. This suggests that the type of radiation had no measurable effect upon the nutritional quality of the protein.

TABLE 3

Average protein efficiency ratios in grams per gram of protein eaten for four week period

Radiation dosage (rad)	Control	Cathode ray	Gamma
None	1.39		
0.28×10^6		1.41	1.48
0.93×10^6		1.39	1.44
2.8×10^6		1.45	1.45
9.3×10^6		1.32	1.36
Average	1.39	1.39	1.43
Standard error of mean	0.19		

These results compare favorably with those reported by Cannon ('59) in the ten week study. The results obtained in this study average slightly higher, in agreement with the time variable suggested by findings of Campbell ('60). Since the protein value of the diets was 12 per cent and a level of 5 per cent fat was used, and the ratios were obtained during a four week study, the variables suggested by Campbell as most desirable for obtaining reliable protein efficiency ratios were apparently adequately controlled in this study. The ratios compare favorably with those obtained by Hove and co-workers ('45) who in assaying protein quality fed a 10 per cent level of protein supplied by commercially blended hard spring wheat, milled and unmilled, to 35 to 45 gram weanling rats for six weeks. The authors found that the protein efficiency values ranged from 0.84 for patent flour to 2.86 for wheat germ. The protein efficiency ratio of whole wheat was 1.40.

It should be noted in reviewing Table 3 that the same trend is observed with regard to the level of irradiation as was suggested in the results of weight gain per gram of food eaten. The lower levels of irradiation when incorporated in diets and fed to animals have resulted in similar protein efficiency ratios. The highest level of irradiation, 9.3×10^6 rad, whether cathode ray or gamma, when incorporated in diets and fed to animals, has resulted in apparently lower protein efficiency ratios. However, the results were not found statistically significant.

Although growth and protein efficiency ratios may give a relative picture of the quality of irradiated foods fed to animals, it was considered advisable to investigate more sensitive measurements of dietary quality. Thus, liver xanthine oxidase, liver cytochrome oxidase activities were determined and the results are presented in Table 4.

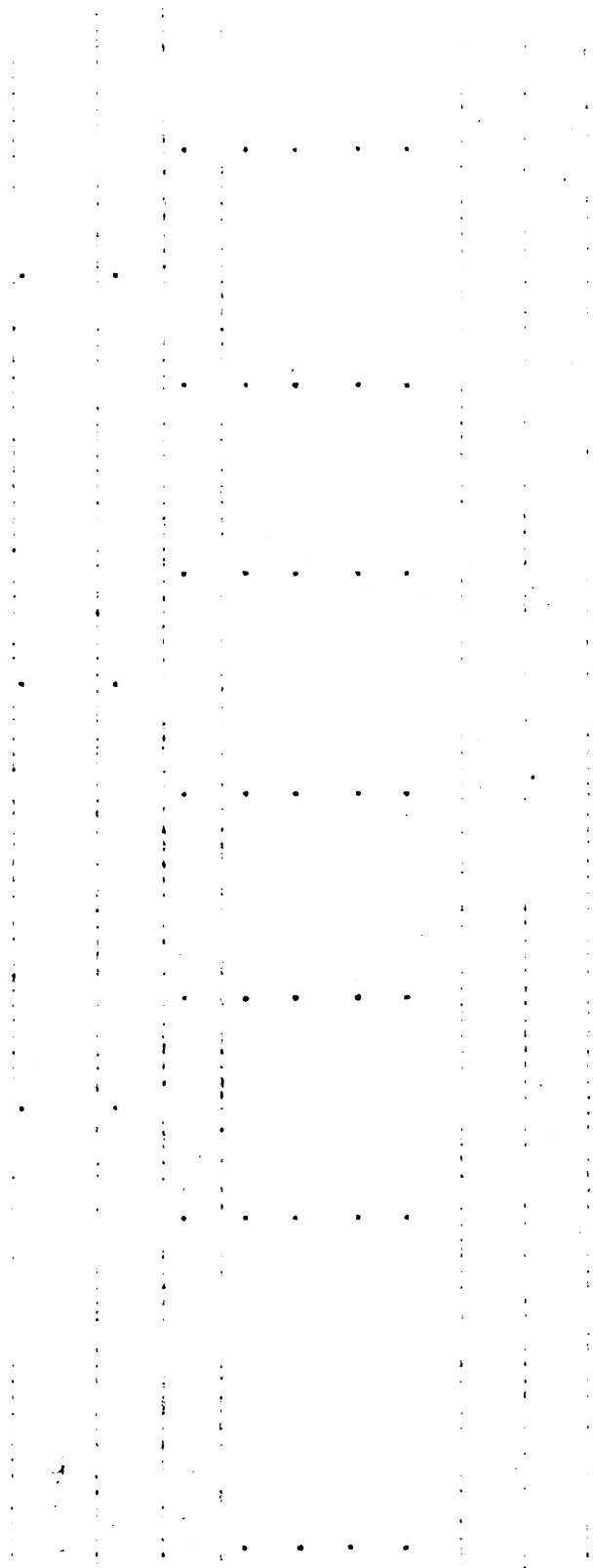
The quantities of these components measured in the livers of animals fed irradiated diets were consistently lower than those obtained from the livers of the control wheat-fed animals, and the depression was most pronounced in those livers from gamma-irradiated wheat-fed animals. The depression was observed in the activities of both the liver xanthine oxidase and the cytochrome oxidase enzyme system. The level of irradiation appeared to have little effect upon the activities measured.

Cannon ('59) assayed only liver xanthine oxidase. The ten week study suggested depression of the liver xanthine oxidase activity also, but the slight depression noted was with both types of radiation and varied inversely with the level of irradiation. The results of this study do not agree with those reported by Read and Kraybill ('58) who

TABLE 4

Average of the liver nitrogen, xanthine oxidase, and cytochrome oxidase activities in the four week study

	Liver nitrogen gm/gm liver		Xanthine oxidase, moles disapp./hour/gm liver		Cytochrome oxidase, seconds^{-1} per gm liver	
Radiation dosage (rad)	Cathode ray	Gamma	Cathode ray	Gamma	Cathode ray	Gamma
0.28×10^6	0.0835	0.0805	4.4	3.9	13.34	11.85
0.93×10^6	0.0825	0.0856	4.4	3.7	12.72	11.26
2.8×10^6	0.0815	0.0819	4.2	3.3	13.39	11.35
9.3×10^6	0.0900	0.0843	4.3	3.9	14.54	10.84
Average	0.0844	0.0831	4.3	3.7	13.50	11.32
Control	0.0885		5.3		14.80	
Standard error of mean	0.0075		1.3		2.2	



found the liver xanthine oxidase activity to be unchanged and the cytochrome oxidase activity increased upon feeding gamma-irradiated diets to rats for four generations. The levels of irradiation reported, however, were not so high as those reported here. Pirie ('56) has suggested that the effect of irradiation upon living cells may be to disturb the internal barrier of the cell so that enzymes may move into sites where they are normally excluded. Thus the immediate activity might appear to increase but as the enzyme was influenced by the unnatural surrounding media, its activity would decrease. Whether this is also possible when cells are metabolizing irradiated foods remains to be seen.

The depression of activity suggested by the results of this study were not found statistically significant when subjected to analysis of variance, and therefore, the T-test was utilized in an effort to compare the over-all effect of feeding cathode ray- and gamma-irradiated wheat diets with that found in the non-irradiated wheat diet-fed animals. (See Appendix, Tables vii and viii). Significance was found by the T-test between the control and the average obtained from the gamma-irradiated wheat-fed animals for both systems, but no significance was found for either enzyme system between the control and the average obtained from animals fed cathode ray-irradiated wheat diets. Although the T-test can only be considered a supplementary appraisal of the trend noted, it was interesting to observe that the average obtained from assay of the livers of animals fed gamma-irradiated wheat diets was responsible for the significance found in the T-test in both enzyme systems.

Table 4 also contains the average liver nitrogen measurements which were not found significantly different by either statistical procedure. (See Appendix, Table v). Miller ('48) and Younathan and co-authors ('56) have both suggested that when the organism is subjected to an unusual physiological condition, such as protein deficiency, that it may tend to first sacrifice those enzymes which will enable the organism to conserve its metabolic economy under the stated conditions. Since analysis of variance showed no significance in the results obtained in either enzyme system, and only questionable significance may be attached to the trend observed until additional data is obtained from further studies, possibly the conservation of metabolic economy noted here is one of depressed activity rather than reduction of actual enzyme protein.

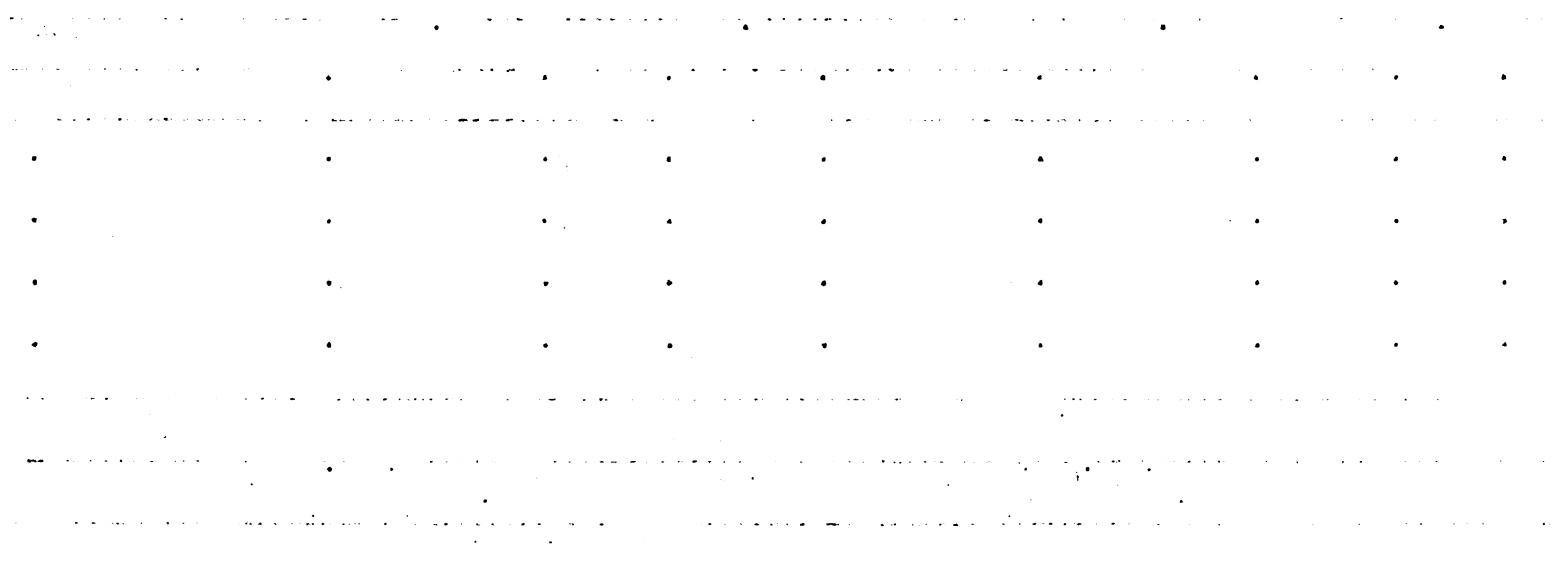
The growth, protein efficiency ratios, and measurements obtained from the enzyme studies of both this study and Cannon's ('59) seem to suggest that there is a difference in wheat quality due to the type of radiation and the level of irradiation used. Variability of results, however, possibly due to the immaturity of the animals has made the trends noted difficult to prove statistically.

Analysis of variance of the liver fat data showed no significance. The liver fat levels (Table 5) obtained were not high in either the animals on the control or the irradiated wheat diets, and they corresponded to those for normal rats reported by Elvehjem ('56). This author used weanling rats maintained on a diet composed of 88 per cent wheat supplying 9.1 per cent protein and after two weeks on the diet, the liver fat was found to be 12 per cent. The fat content of the livers

TABLE 5

Comparison between averages of xanthine oxidase and per cent of liver fat in the four week and the ten week studies

	Four week study				Ten week study			
	Xanthine oxidase, moles disapp./hr./gm liver		% fat/gm liver		Xanthine oxidase, moles disapp./30.min./gm liver		% fat/gm liver	
Radiation dosage (rad)	Cathode ray	Gamma	Cathode ray	Gamma	Cathode ray	Gamma	Cathode ray	Gamma
0.28×10^6	4.4	3.9	8.81	8.31	11.9	11.1	4.8	6.8
0.93×10^6	4.4	3.7	8.28	8.79	10.6	9.1	5.39	6.09
2.8×10^6	4.2	3.3	10.09	8.12	10.1	10.4	5.69	6.28
9.3×10^6	4.3	3.9	8.87	9.20	9.2	12.2	8.25	6.02
Average	4.3	3.7	9.01	8.60	10.45	10.2	6.03	6.29
Control	5.3		8.81		12.2		3.50	



in this study averaged between 8.12 per cent and 10.09 per cent (Standard error of the mean, 2.8). The high average noted in the 9.3×10^6 rad cathode ray diet animals was due to one animal in this diet group who showed 20.79 per cent liver fat. The high percentage found upon ether extraction was substantiated upon examination of the histological sections of this liver. (See Appendix, Tables vi and ix).

In comparing the liver fat obtained in this four week study with that found by Cannon ('59) in which progressively increasing liver fat levels varied directly with increasing irradiation levels, it should be noted that the results reported here compare with the highest level reported by Cannon. Also, the relationship between the liver xanthine oxidase activity and the per cent of liver fat observed by Cannon was not found in this study.

Although the data obtained does not bear out the type of adaptation to an amino acid imbalance reported by Carroll ('60) and suggested by Cannon ('59) as a possible factor in that study, adaptation still can not be completely ruled out on the basis of the present findings. It is possible that the adaptation is of another nature. In the involved picture of dietary protein, the liver and fat metabolism, the accumulation of fat tends to follow the removal of protein from the liver cell. It is possible that irradiation of the wheat slightly altered the protein structure and in turn altered the metabolism of it by the liver cell. Whereas the animals upon the control diet were able to adjust to the limitations of wheat protein, it is suggested that the animals on the irradiated wheat protein diets were not able to do this as readily. It would be advisable to undertake a long term study, the nature of which could attempt to determine at regular intervals over

a long period of time, the various changes noted in these two studies. Not until adaptation of some nature is clearly ruled out can it be taken from the realm of possibility.

It was hoped in using the 12 per cent level of dietary protein that the wheat protein and its limiting amino acid lysine would be sufficiently borderline so that any damage to lysine induced by the irradiation would be detected by the animals. The histological study was undertaken to see if the animal was able to detect any difference in the protein which would result in the indirect effect of altered fat deposit within the cell and lobule of the liver itself.

In reading the histological slides, some point of reference had to be established. Two were chosen from non-irradiated wheat diets, both representative of the average, one coming from the 12 per cent protein diet and the other from the 7 per cent protein diet. Each of the individual animal tissues was compared with both of these in regard to the pattern of fat deposition. In addition, each of the animal tissues was compared with the 12 per cent wheat control animal tissue for the amount of fat present, and this was reported as one, two, three, and four plus. (See Appendix, Table ix). The 12 per cent control was ranked as zero.

In general, the individual histological estimate of amount of fat agreed with the other extractions. In comparing the fat deposition of each against the 12 per cent and 7 per cent control wheat-fed animal tissues, a picture of a possible intensified dietary unbalance, particularly in the gamma-irradiated wheat diet animals evolved.

Photomicrographs of representative tissues are presented in Plates I, II, and III.

PLATE I

Histological sections of liver from animals fed 12 per cent and 7 per cent wheat diets.

Oil Red O Fat stain

Figure 1. Twelve per cent protein diet

The tissue exhibited predominately portal deposition of the fat. Only fine droplets of fat were observed and these tended to be located at the ends of the portal areas. Magnification, 1000 X

Figure 2. Seven per cent protein diet

Fine to medium droplets of fat were observed in the cells of the portal areas and in the intermediate zones. Fat-rich cells were scattered throughout the areas. Magnification, 370 X

PLATE I

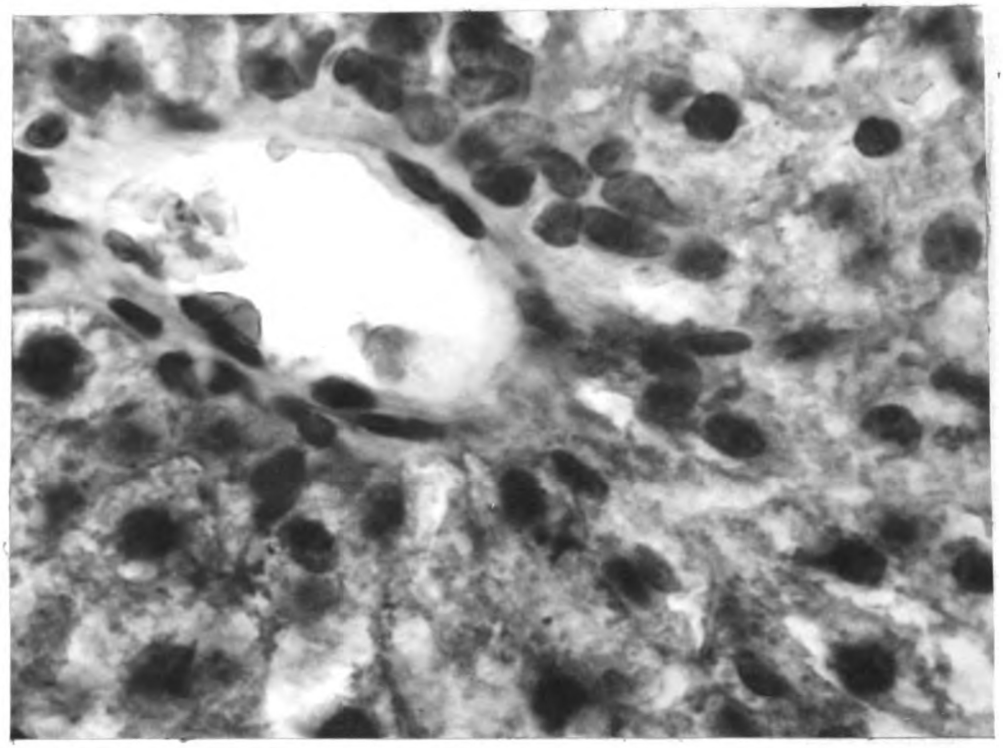


Figure 1.

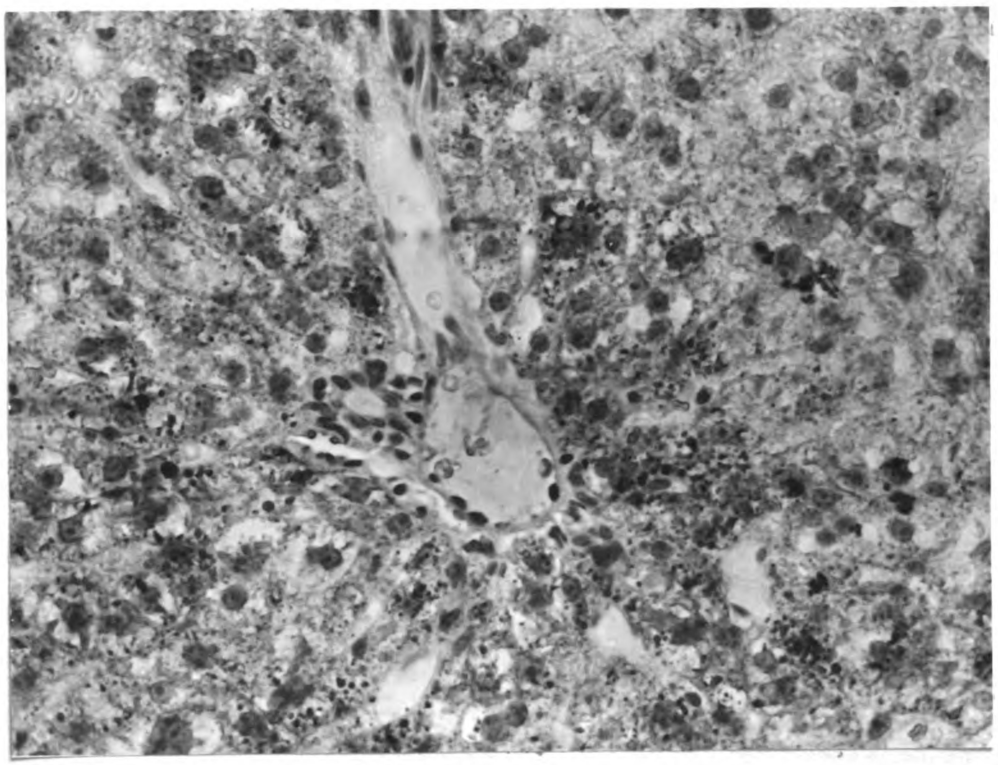


Figure 2.

PLATE II

Histological sections of liver from animals fed gamma- and cathode ray- irradiated wheat diets.

Oil Red O Fat stain

Figure 1. Gamma-irradiated wheat, 9.3×10^6 rad

Fine droplets of fat were observed in individual cells scattered throughout the lobules. No definite pattern could be established.

Magnification, 390 X

Figure 2. Cathode ray-irradiated wheat, 2.8×10^6 rad

Medium to large droplets of fat were observed in cells of the portal areas and intermediate zone. The fat-rich cells were scattered throughout the areas and some large single nucleoli were noted.

Magnification, 360 X

PLATE II

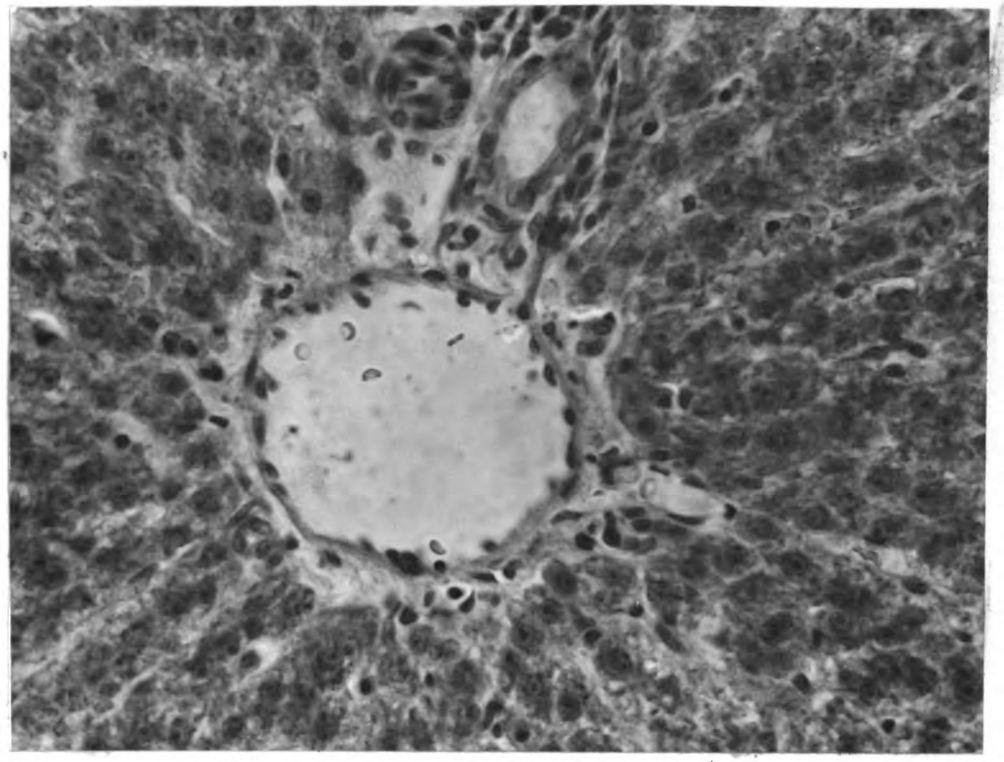


Figure 1.

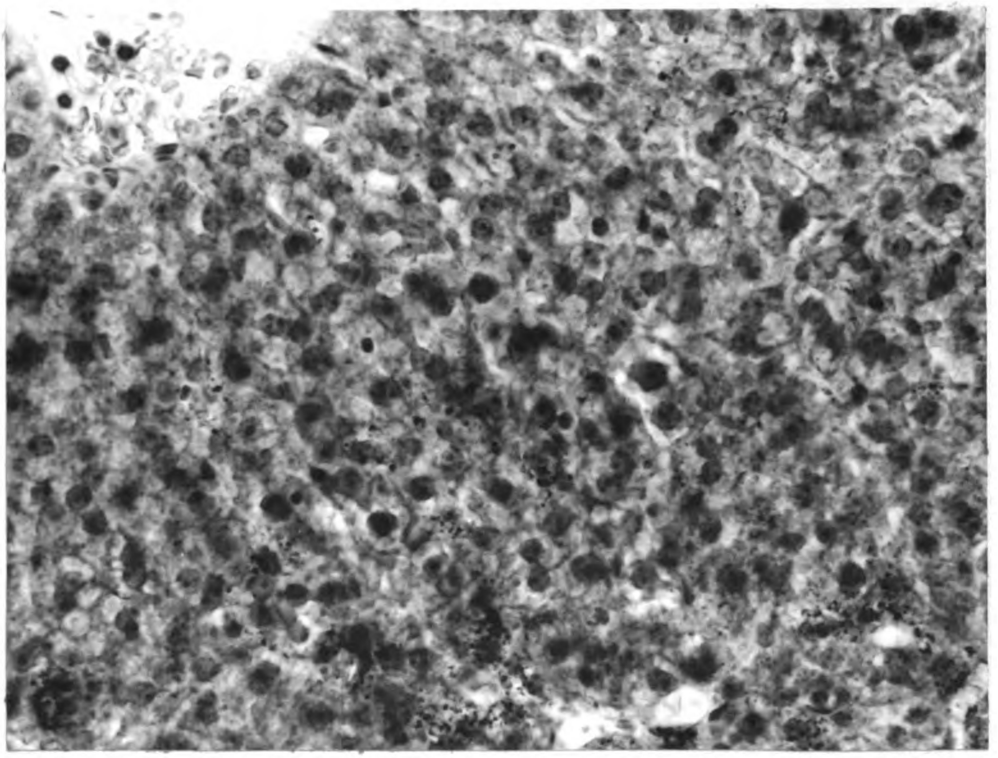


Figure 2.

PLATE III

Histological sections of liver from animals fed gamma- and cathode ray-irradiated wheat diets.

Oil Red O Fat stain

Figure 1. Gamma-irradiated wheat, $.93 \times 10^6$ rad
Fine to large droplets of fat were observed in cells surrounding the portal areas. Some fat-rich cells were scattered into the intermediate zone and the central vein area. Magnification, 365 X

Figure 2. Cathode ray-irradiated wheat, 9.3×10^6 rad
Fine to large droplets of fat-rich cells were massed in the portal areas, intermediate zone, and the central vein area. Heavy fat deposition was noted throughout the tissue. Magnification, 330 X

PLATE III

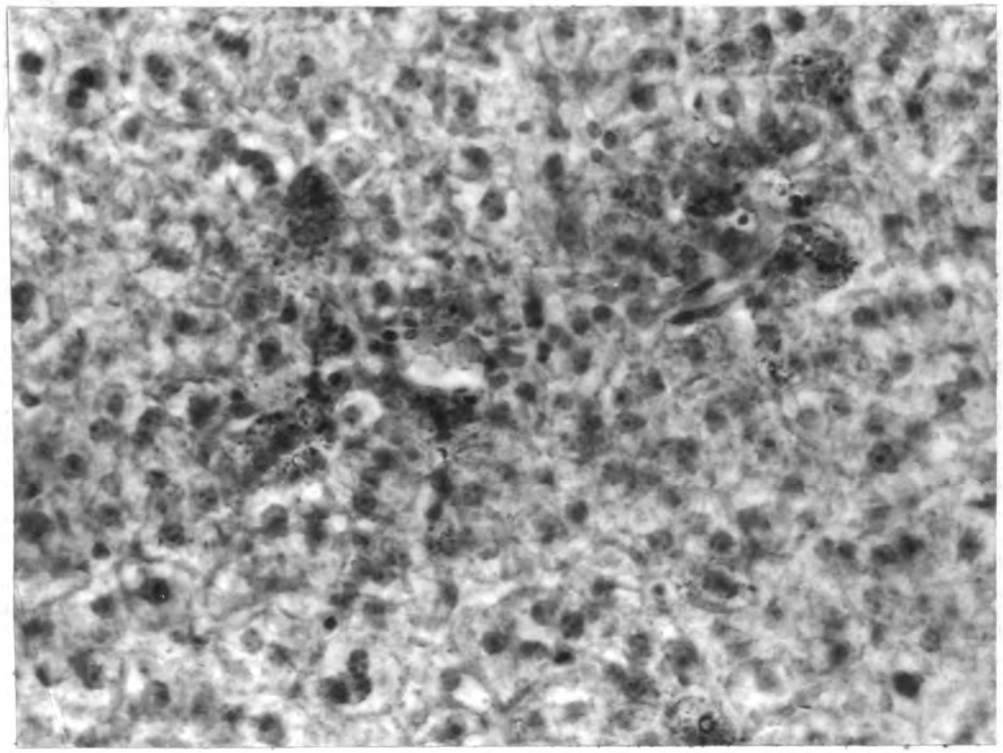


Figure 1.

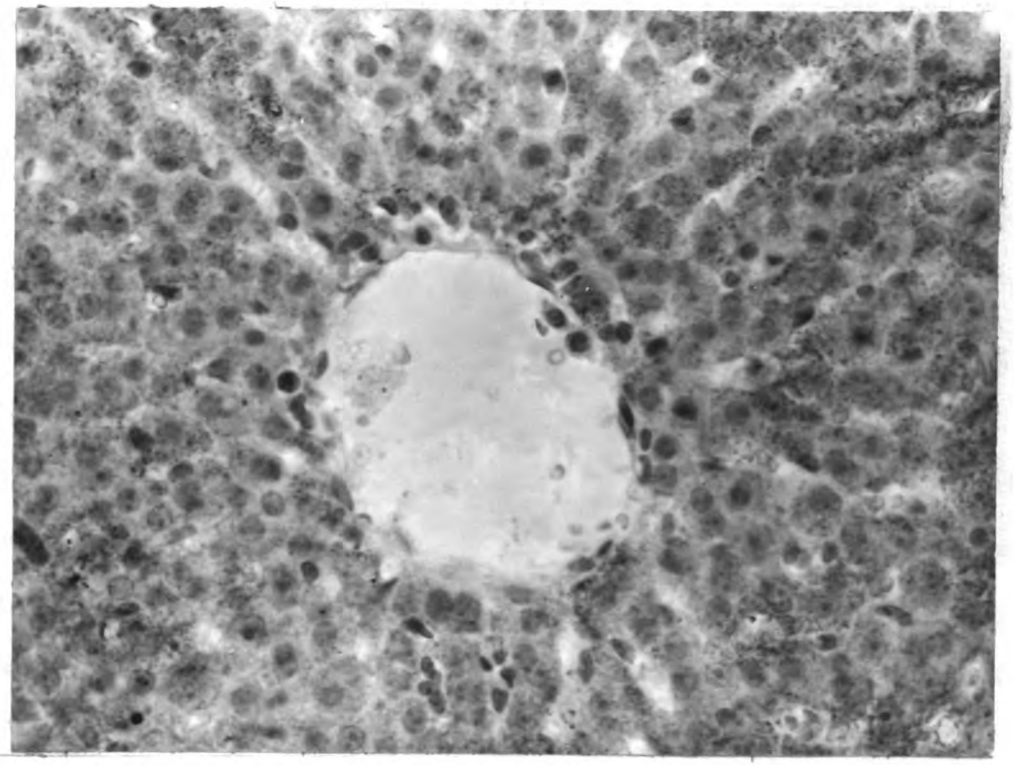


Figure 2.

There appeared to be a difference in the way in which fat was deposited within the liver lobule between the tissues obtained from feeding the cathode ray- and gamma-irradiated diets and the 12 per cent control wheat diet. All the tissues exhibited predominately portal deposition of the fat in agreement with Shils and co-workers ('54) and Adamstone and Spector ('50). As more fat was moved into the lobule, rather than being found at one end of a portal area as the control showed, the fat became periportal. Then from the immediate periportal deposit, it fanned out around and away from the portal areas and along the periphery of the lobule. As more and more fat arrived in the lobule, the heaviest deposits were still seen in the portal areas but now individual fat cells were found in the intermediate zone and on rare occasion in the central vein area as well. The fat droplets themselves varied from a fine peppery size to large droplets. The nuclei did not appear to undergo any changes other than being pushed to the side of the cell. There were one to four nucleoli present in the nuclei with occasional large ones as Adamstone and Spector ('50) showed.

The above changes noted in the fat pattern have been suggested in studies discussed earlier in which phenylalanine, leucine, histidine, tryptophane, methionine, threonine, and lysine deficiencies have been individually studied. Of all the amino acid deficiencies, lysine appears to be the most passive in nature and slowest to show fat-pattern changes. It has been well established as the major limiting amino acid for wheat. In the study reported by Harris and Burress ('59), fatty livers were found in animals maintained on an 8 per cent cereal protein diet from 14 to 28 days. The animals maintained on a 15 per cent protein diet for the same period of time did not show fatty liver. When

the 8 per cent diet was supplemented with varying levels of lysine, the fatty livers were prevented. It follows that the 7 per cent level of wheat protein used in this study might be expected to show fatty livers induced by the limiting amino acid, lysine.

The fat cells found in the sections made from the liver of the animal fed the 7 per cent non-irradiated wheat diet had a characteristic impact upon the observer. Fine to medium-sized droplets were found in individual cells following the portal areas, fanning around them and over the periphery. Individual cells were found in the intermediate zone and occasionally in the central vein area. There was a clumped scattered appearance to the fat-rich cells in contrast to the smooth steady movement of fat cells observed in agreement with the 12 per cent control. Dick and co-workers ('52) and Vennart and co-workers ('58) have reported changes similar to these when diets deficient in lysine were fed.

When the tissues derived from the animals fed various levels of cathode ray- and gamma-irradiated wheat were compared with the 7 per cent control, the gamma-irradiated tissues seemed to have a generalized scattering of fat-rich cells with 12 out of 31 tissues comparable with the 7 per cent control. And of the 12 tissues, ten were from the two lowest levels of gamma-irradiated diets, 0.28×10^6 and 0.93×10^6 rad. The upper levels, 2.8×10^6 rad and 9.3×10^6 rad had a scattered appearance but were so diffuse that no definite pattern could be discerned. The cathode ray-irradiated diet-derived-tissues, however, compared more closely with the 12 per cent control tissue and only eight of their total number of 32 showed any characteristics of the 7 per cent

control. The eight tissues were evenly distributed among the various levels. These tissues do not represent a majority of the tissues examined. However, this does not preclude the possibility of an amino acid unbalance intensified by irradiation.

No explanation for the majority of changes in the tissues derived from feeding the lower levels of gamma-irradiated wheat diets can be made since the source of irradiation was the same. A more uniform pattern of fat deposition as was found in the tissues derived from cathode ray-irradiated wheat diet animals was to be expected. The tissues from the gamma-irradiated wheat-fed animals as a whole had a more scattered clumped appearance. The tissues from the cathode ray-irradiated wheat-fed animals gave a clearer, smoother picture of fat deposition. It is possible that a long term feeding study would clarify the reason for this, and whether the trend in fat deposition noted here is transitory or characteristic.

There was a consistent trend throughout the results reported in this study and in the findings of Cannon ('59) indicating a possible protein alteration of some nature. The structural damage which may be induced by irradiation and the slowness of response of an animal to lysine deficiency as noted earlier may well be an explanation for this consistent trend. It would appear from the findings reported here that the type of radiation had more influence than the level of irradiation used. Since only the initial attack of cathode ray- and gamma-irradiation is thought to be different, the nature of this difference should be further investigated.

In general, the animals liked the irradiated diets, ate them, and gave no outward signs of being other than healthy, growing young animals. It remains to be determined whether the changes observed in the liver tissues are detrimental to the well-being of the whole animal.

SUMMARY AND CONCLUSIONS

Seventy-six male weanling albino rats were allotted at random to individual wire-bottomed cages and fed food and water ad libitum for a four week period. Wheat was divided into nine lots, one of which was not exposed to radiation and served as a control. Of the remaining eight lots, four were exposed to cathode ray and the other four to gamma-irradiation, each at the following levels: 0.28×10^6 rad, 0.93×10^6 rad, 2.8×10^6 rad, and 9.3×10^6 rad. The wheat was ground following irradiation, incorporated into diets to provide a total of 12 per cent protein, and eight animals were maintained on each of the nine diets. A third group of four animals was fed a non-irradiated wheat diet of 7 per cent protein as a supplementary control. Records of weekly weight changes and daily records of food consumption were kept. On the last four days of the experimental period, one rat from each diet group was sacrificed by decapitation, the liver removed, and xanthine oxidase and cytochrome oxidase activities determined. One lobe of each liver was placed in 10 per cent formaldehyde for histological examination, and the remainder of each liver was saved for nitrogen and fat determinations. Protein efficiency ratios and growth measurements were evaluated. All findings were subjected to analysis of variance, and in addition, measurements of the enzyme system activity were subjected to T-testing.

There appeared to be no difference in the feed intakes and weight gains due to the type of radiation but there was indication that the level of irradiation had some effect. The animals tended to grow at the same rate except for those on the highest level of irradiation who appear-

ed to grow somewhat more slowly. The same trend persisted when the average weight gains per gram of diet eaten were calculated. Protein efficiency ratios were found to be similar in animals fed the lower levels of irradiation but again, the highest level of irradiation, regardless of the type incorporated into diets, resulted in lower protein efficiency ratios.

The activities of xanthine oxidase and cytochrome oxidase measured in the livers of animals fed irradiated diets were consistently lower than those obtained from the livers of control wheat-fed animals. The depression of activity of both enzyme systems was most pronounced in livers from gamma-irradiated wheat-fed animals, and was found statistically significant at the 5 per cent level with the T-test but not with analysis of variance. Histological examination of fat deposition within the cell and lobule of the individual livers presented a picture of possible intensified dietary unbalance, especially in the gamma-irradiated wheat-fed animals. The level of irradiation appeared to have little effect upon the degree of depression noted in the enzyme system activities and upon the fat deposition noted histologically.

The findings suggest that the type of radiation may have more significant influence than the level of irradiation with regard to the animal's ability to metabolize the food presented. There are indications in the findings of this study that some type of alteration has been produced in the nutritional value of the wheat by the irradiation process. Since wheat protein is nutritionally incomplete, assay of the irradiated wheat for the limiting amino acids might clarify one possible aspect of the alteration. In addition, a long term feeding study, with animals being sacrificed at regular intervals, might clarify whether the fat

deposition noted was transitory or characteristic and potentially detrimental to the well-being of the animals.

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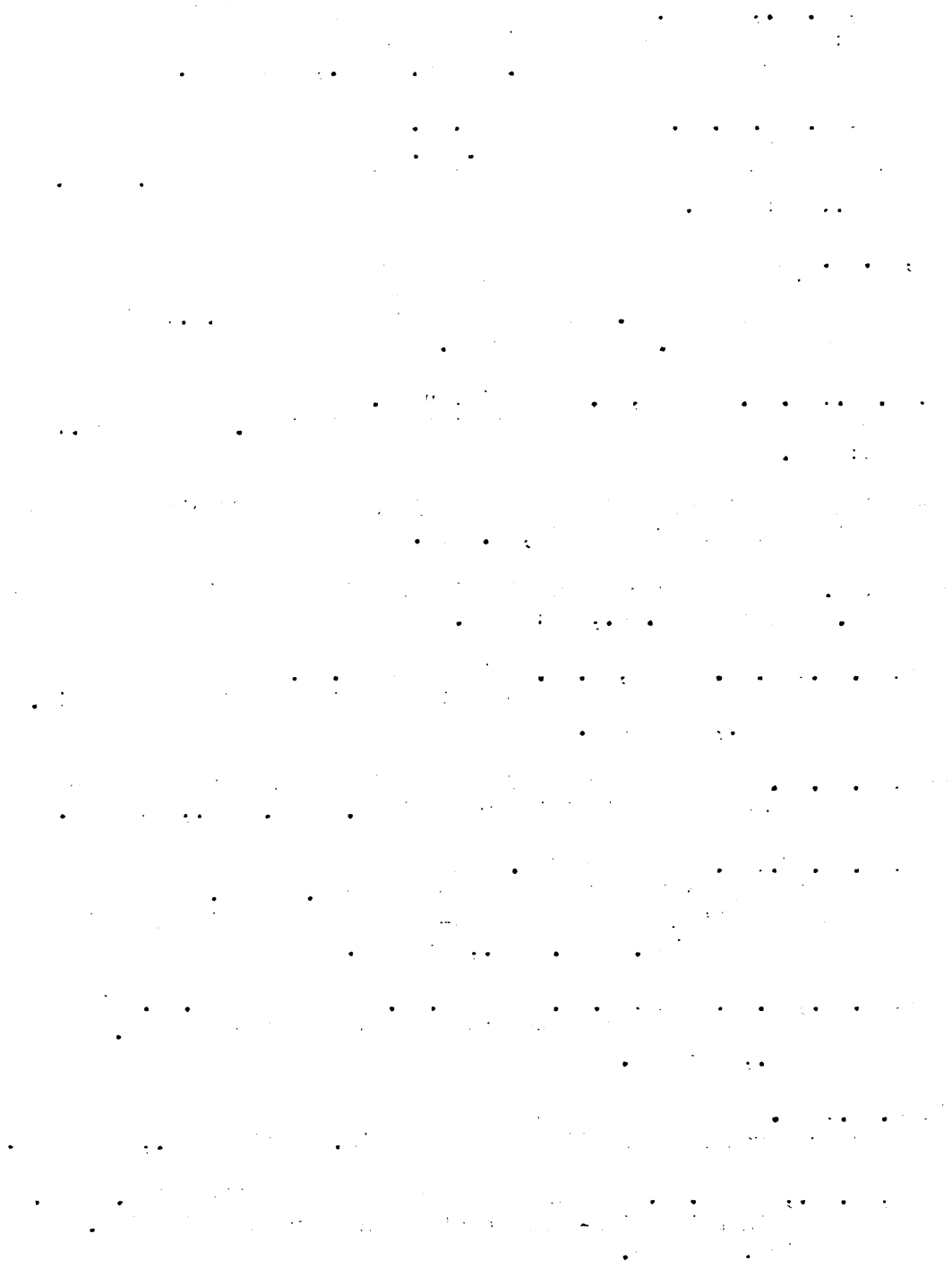

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APPENDIX

HISTOLOGICAL METHOD

The liver lobe used for histological examination was cut from the intact liver before taking the amount necessary for the enzyme and chemical studies. The same lobe was taken from all animals. It was the small tear-drop caudate lobe situated to the left beneath the two upper large lobes, and varied in weight from 0.32 to 0.80 grams. Each lobe was quickly placed in ten volumes of 10 per cent formalin neutralized with marble chips. They were allowed to fix for a minimum of two months and were creamy white in color.

Tissue was prepared for cutting by gelatin infiltration (Gurr, '56). The lobe was cut into two or three lengthwise pieces depending upon its over-all size. These in turn were cut in half, crosswise. An attempt was made in every liver to secure the same middle, lower-half of the lobe for infiltration. The tissues were rinsed in running water and immersed in 5 per cent gelatin solution for 24 hours in a 37° incubator. Following this, they were immersed in 10 per cent gelatin for 12 hours, 15 per cent gelatin for 6 hours, both in 37° incubator, and then embedded in 20 per cent gelatin and allowed to set overnight in the refrigerator. The next day the tissues were trimmed from the gelatin blocks and immersed in 10 per cent formalin for 24 hours, after which they were ready for cutting. Although the time involved in gelatin infiltration was considerable, sections could be cut at five microns and handled with each throughout the staining and mounting techniques.

The tissues were washed in running water for ten minutes prior to freezing. They were frozen with carbon dioxide and cut at five microns on a Spencer Automatic Clinical Microtome with freezing attachment. The sections were stained with Oil Red O following the technique of Bell, ('59). Since the gelatin was not removed prior to staining, the free sections were allowed to remain in the Oil Red O from six to sixteen hours. No appreciable loss of fat was noted and an intense stain was obtained. The free sections were counter-stained one minute with Groat's hematoxylin and blued for ten minutes with 1 per cent sodium bicarbonate. After rinsing in distilled water, they were fixed to the slides with Mayer's albumin and mounted with glycerine jelly. The slides were allowed to dry for one month before final cleaning.

TABLE i - WEIGHT GAIN IN GRAMS

		CATHODE RAY RADIATION (RAD)				GAMMA RADIATION (RAD)			
GROUP	CONTROL	0.28×10^6	0.93×10^6	2.8×10^6	9.3×10^6	0.28×10^6	0.93×10^6	2.8×10^6	9.3×10^6
PERIOD I	35	34	17	32	24	32	29	35	26
	28	28	19	35	27	36	27	23	40
	25	27	36	28	24	33	29	29	34
	31	34	36	32	39	25	30	28	36
PERIOD II	24	38	44	42	40	43	45	42	17
	26	39	41	37	24	46	46	46	44
	21	34	44	53	39	53	47	42	28
	18	40	40	38	39	38	32	51	43
AVERAGE OF TYPE	30		34.5				36.0		
GRAND MEAN	30	34	35	37	32	38	36	37	33

TABLE ii - FEED INTAKE IN GRAMS

		CATHODE RAY RADIATION (RAD)				GAMMA RADIATION (RAD)			
GROUP	CONTROL	0.28×10^6	0.93×10^6	2.8×10^6	9.3×10^6	0.28×10^6	0.93×10^6	2.8×10^6	9.3×10^6
PERIOD I	188	214	186	189	195	207	192	206	180
	186	158	173	229	196	198	215	173	205
	192	212	201	180	171	210	176	189	205
	211	209	214	216	227	170	197	192	218
PERIOD II	192	201	192	222	199	229	233	204	147
	210	220	227	203	196	229	202	225	248
	189	185	224	251	226	240	251	234	175
	193	224	222	208	200	221	178	261	242
AVERAGE OF TYPE	191		205				208		
GRAND MEAN	191	203	205	212	201	213	205	210	202

TABLE iii - WEIGHT GAIN PER GRAM OF FOOD EATEN

		CATHODE RAY RADIATION (RAD)				GAMMA RADIATION (RAD)			
GROUP	CONTROL	0.28×10^6	0.93×10^6	2.8×10^6	9.3×10^6	0.28×10^6	0.93×10^6	2.8×10^6	9.3×10^6
PERIOD I	.186	.159	.091	.169	.123	.155	.151	.170	.144
	.151	.176	.109	.152	.138	.182	.125	.134	.195
	.130	.127	.179	.155	.141	.158	.164	.153	.166
	.146	.163	.167	.148	.171	.147	.153	.146	.165
PERIOD II	.141	.188	.229	.189	.202	.188	.192	.206	.117
	.168	.177	.180	.183	.122	.201	.228	.205	.177
	.201	.183	.196	.211	.172	.220	.187	.179	.159
	.141	.179	.181	.182	.195	.173	.181	.195	.177
AVERAGE	.158	.169	.166	.174	.158	.178	.173	.173	.162
AVERAGE OF TYPE	.158		.167				.171		

ANALYSIS OF VARIANCE

SOURCES	DF	MEAN SQ.	RATIO
TOTAL	71	---	
DIETS	8	.0005	0.833
PERIODS	1	.0177	29.5**
REMAINDER	62	.0006	

**Significance at the 5% level

TABLE iv - PROTEIN EFFICIENCY RATIOS, EXPRESSED AS WEIGHT GAIN IN GRAMS PER GRAM OF PROTEIN CONSUMED

		CATHODE RAY RADIATION (RAD)				GAMMA RADIATION (RAD)			
GROUP	CONTROL	0.28×10^6	0.93×10^6	2.8×10^6	9.3×10^6	0.28×10^6	0.93×10^6	2.8×10^6	9.3×10^6
PERIOD I	1.55	1.33	0.76	1.41	1.02	1.29	1.26	1.42	1.20
	1.26	1.47	0.91	1.26	1.15	1.52	1.05	1.11	1.62
	1.08	1.06	1.50	1.30	1.18	1.32	1.37	1.28	1.39
	1.22	1.35	1.39	1.24	1.43	1.23	1.28	1.21	1.39
PERIOD II	1.75	1.57	1.89	1.57	1.68	1.57	1.60	1.73	0.98
	1.39	1.49	1.50	1.52	1.02	1.67	1.91	1.70	1.34
	1.68	1.63	1.64	1.76	1.43	1.83	1.56	1.50	1.48
	1.17	1.49	1.51	1.51	1.62	1.43	1.50	1.63	1.47
AVERAGE	1.39	1.41	1.39	1.45	1.32	1.48	1.44	1.45	1.36
AVERAGE OF TYPE	1.39		1.39				1.43		

ANALYSIS OF VARIANCE

SOURCES	DF	MEAN SQ.	RATIO
TOTAL	71	---	
DIETS	8	0.021	0.55
PERIODS	1	1.34	35.26**
REMAINDER	62	0.038	

**Signicifance at the 5% level

TABLE V - PERCENTAGE OF NITROGEN IN LIVER (DRY WEIGHT)

		CATHODE RAY RADIATION (RAD)				GAMMA RADIATION (RAD)			
GROUP	CONTROL	0.28×10^6	0.93×10^6	2.8×10^6	9.3×10^6	0.28×10^6	0.93×10^6	2.8×10^6	9.3×10^6
PERIOD I	7.23	7.84	—*	7.63	9.78	7.77	7.98	9.23	9.08
	8.71	8.56	8.31	7.78	7.48	6.84	8.63	7.89	9.27
	8.54	8.04	7.69	5.99	9.31	8.20	9.43	7.91	8.04
	8.69	9.78	7.66	8.86	9.81	—*	8.61	8.31	8.44
PERIOD II	8.51	8.01	8.50	8.37	8.62	—*	8.78	7.15	7.55
	8.96	8.58	8.58	9.25	10.01	8.39	7.46	7.87	7.77
	10.01	7.96	8.69	8.30	8.50	8.47	8.65	8.15	8.90
	10.18	8.06	8.32	9.01	8.53	8.64	8.93	9.00	8.41
AVERAGE	8.85	8.35	8.25	8.15	9.00	8.05	8.56	8.19	8.43
AVERAGE OF TYPE	8.85		8.44				8.31		

*Insufficient liver

ANALYSIS OF VARIANCE

SOURCES	DF	MEAN SQ.	RATIO
TOTAL	68	——	
DIETS	8	.00007	1.25
PERIODS	1	.0000	.00
REMAINDER	59	.000056	

TABLE vi - PERCENTAGE OF FAT IN LIVER (DRY WEIGHT)

		CATHODE RAY RADIATION (RAD)				GAMMA RADIATION (RAD)			
GROUP	CONTROL	0.28×10^6	0.93×10^6	2.8×10^6	9.3×10^6	0.28×10^6	0.93×10^6	2.8×10^6	9.3×10^6
PERIOD I	6.73	12.80	—*	14.11	6.80	8.81	10.51	11.92	11.59
	10.94	7.99	6.21	4.50	5.69	8.99	8.33	8.91	11.50
	9.93	7.50	5.14	6.22	4.76	6.50	7.64	7.00	5.45
	10.25	9.33	7.71	13.46	7.67	—*	6.92	6.12	7.43
PERIOD II	10.47	6.09	9.69	7.22	7.62	9.21	—*	6.83	10.01
	6.30	10.05	7.17	14.76	20.79	4.47	9.47	6.79	10.01
	9.34	9.82	13.40	6.99	8.25	9.70	9.39	6.78	9.65
	6.56	6.91	8.61	13.48	9.40	9.98	9.25	10.64	7.97
AVERAGE	8.81	8.81	8.28	10.09	8.87	8.31	8.79	8.12	9.20
AVERAGE OF TYPE	8.81		9.01				8.60		

*Insufficient liver

ANALYSIS OF VARIANCE

SOURCES	DF	MEAN SQ.	RATIO
TOTAL	68	——	
DIETS	8	2.74	0.333
PERIODS	1	12.52	1.523
REMAINDER	59	8.22	

TABLE vii - LIVER XANTHINE OXIDASE ACTIVITY, EXPRESSED AS MICROMOLES OF XANTHINE DISAPPEARING PER HOUR PER GRAM OF LIVER (WET WEIGHT)

		CATHODE RAY RADIATION (RAD)				GAMMA RADIATION (RAD)			
GROUP	CONTROL	0.28×10^6	0.93×10^6	2.8×10^6	9.3×10^6	0.28×10^6	0.93×10^6	2.8×10^6	9.3×10^6
PERIOD I	5.2	3.0	6.6	4.4	3.7	2.9	3.6	2.6	2.4
	4.2	8.7	3.1	3.3	3.8	3.3	3.4	4.4	4.3
	5.0	2.7	4.0	3.0	4.7	4.1	4.3	2.0	2.8
	3.9	4.4	4.9	6.2	4.1	6.9	4.3	3.9	4.2
PERIOD II	4.9	4.9	3.4	2.0	3.0	4.5	3.4	2.2	5.2
	6.3	2.2	3.4	6.2	7.9	3.0	3.0	4.0	4.5
	5.8	5.9	4.4	4.1	3.5	2.9	2.9	3.5	4.8
	7.0	3.5	5.5	4.1	3.5	4.0	4.5	3.7	3.2
AVERAGE	5.3	4.4	4.4	4.2	4.3	3.9	3.7	3.3	3.9
AVERAGE OF TYPE	5.3		4.3				3.7		

ANALYSIS OF VARIANCE

SOURCES	DF	MEAN SQ.	RATIO
TOTAL	71	——	
DIETS	8	2.51	1.43
PERIODS	1	.09	.05
REMAINDER	62	1.76	

T TEST

$$T = \frac{5.3 - \left[\frac{4.3 \quad 3.7}{2}\right]}{\sqrt{\frac{1.76}{8}} + \sqrt{\frac{1.76}{8}}}$$

T = 2.6**

T for cathode ray versus control diets: 1.9

T for gamma versus control diet: 3.9 **

** Significant at the 5% level

TABLE viii - CYTOCHROME OXIDASE ACTIVITY, EXPRESSED IN SECONDS $^{-1}$ AS FIRST ORDER VELOCITY CONSTANT PER GRAM OF LIVER (WET WEIGHT)

		CATHODE RAY RADIATION (RAD)				GAMMA RADIATION (RAD)			
GROUP	CONTROL	0.28×10^6	0.93×10^6	2.8×10^6	9.3×10^6	0.28×10^6	0.93×10^6	2.8×10^6	9.3×10^6
PERIOD I	14.96	10.43	16.49	14.12	——*	12.95	16.98	——*	——*
	16.10	——*	16.89	8.64	12.56	11.91	11.73	11.49	——*
	20.72	15.64	9.24	23.30	15.47	——*	——*	13.54	6.03
	7.49	9.53	12.71	15.92	13.56	——*	11.54	8.52	6.80
PERIOD II	14.93	18.39	11.94	12.29	13.08	14.00	9.71	10.49	11.19
	13.62	9.27	15.85	9.21	20.84	13.25	10.13	9.63	8.20
	15.97	7.40	11.80	11.54	17.40	10.31	8.61	13.97	10.79
	14.64	22.74	6.84	12.13	8.93	8.70	10.13	11.82	22.01
AVERAGE	14.80	13.34	12.72	13.39	14.54	11.85	11.26	11.35	10.84
AVERAGE OF TYPE	14.80		13.50				11.32		

*Insufficient liver

ANALYSIS OF VARIANCE - UNWEIGHED AVERAGES METHOD

SOURCES	DF	MEAN SQ.	RATIO
TOTAL	62	—	
PERIODS	1	.01	.002
DIETS	8	5.16	1.073
P X D	8	5.61	1.166
REMAINDER	45	4.81	

T TEST

$$T = \frac{14.80 - \left[\frac{13.50 + 11.32}{2}\right]}{\sqrt{\frac{4.81}{8}} + \sqrt{\frac{4.81}{56}}}$$

T = 2.88**

T for cathode ray versus control diets: 1.5

T for gamma versus control diets: 3.9**

** Significant at the 5% level

TABLE ix - SCORING OF HISTOLOGICAL SECTIONS FOR FAT AND POSSIBLE AMINO ACID UNBALANCE

	12% WHEAT DIET		7% WHEAT DIET	
Controls	Scoring	Unbalance	Scoring	Unbalance
	0.5^+	none	4^+	suggestive
	2^+	none	1^+	suggestive
	3^+	none	—*	
	1.5^+	none	—*	
	zero	none		
	CATHODE RAY RADIATION (RAD)		GAMMA RADIATION (RAD)	
	Scoring	Unbalance	Scoring	Unbalance
0.28×10^6	4^+	none	1^+	none
	3^+	suggestive	4	suggestive
	2.5^+	questionable	1	none
	2^+	suggestive	zero	none
	1^+	none	3^+	suggestive
	1.5^+	none	zero	none
	2.5^+	suggestive	2	suggestive
	zero	none	2	suggestive
0.93×10^6	1^+	none	3^+	suggestive
	1^+	none	2.5	suggestive
	1^+	suggestive	0.5	none
	2^+	none	3	suggestive
	1	none	0.5	none
	1	none	0.5	suggestive
	1.5	none	2	suggestive
	1	none	1.5	suggestive
2.8×10^6	3^+	suggestive	0.5^+	none
	1^+	none	3	questionable
	1^+	none	1	none
	1^+	none	1	none
	4	suggestive	0.5	none
	0.5	none	1.5	none
	zero	none	1	none
	zero	none	3	suggestive
9.3×10^6	1	none	2.5^+	suggestive
	zero	none	2	none
	zero	none	1	none
	0.5	none	1	none
	1	none	1	none
	4	complete infil-	1.5	none
	1	none tration	1	none
	3	suggestive	—*	—

*insufficient liver

TABLE x - DATA COMPILED FOR THE 7% WHEAT DIET

	Weight gain in grams	Feed intake in grams	Weight gain per gram of food eaten
	24	192	0.125
	26	210	0.124
	21	189	0.110
	18	193	0.092
AVERAGE	22.5	196	0.113

	Protein Efficiency ratio (grams)	Percentage of nitrogen (dry weight)	Percentage of fat (dry weight)
	1.77	6.90	11.73
	1.78	8.04	9.66
	1.58	8.00	10.17
	1.33	6.85	5.78
AVERAGE	1.61	7.45	9.36

	Liver xanthine oxidase activity, micromoles of xanthine disappearing per hour per gram of liver (wet weight)	Cytochrome oxidase activity, in seconds^{-1}, first order velocity constant per gram of liver (wet weight)
	2.2	6.04
	2.1	7.07
	2.8	8.38
	3.2	13.76
AVERAGE	2.6	8.81

ROOM USE ONLY