THE EFFECT OF HIGH VOLTAGE CATHODE RAY IONIZING RADIATION ON THE BIOLOGICAL VALUE OF WHEAT PROTEIN

By

CATHARINA P. MELEHY

AN ABSTRACT

Submitted to the College of Home Economics of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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ABSTRACT

Each of four lots of ground wheat received high voltage cathode ray ionizing radiation in dosages ranging from 50,000 to 1,000,000 rep. A fifth lot was not irradiated and used as a control. To determine the nutritive value of the protein, each of the five wheat samples was supplemented with vitamins and minerals and fed to eight young adult albino rats. A period of adjustment to the diet of seven days was followed by two succeeding periods, each of seven days, during which urine and feces collections were made and food intake and changes in body weight were recorded. Total nitrogen of food and excreta was determined for each seven day collection period. Protein efficiency ratio, biological value and creatinine nitrogen percentage in the urine were calculated to evaluate the nutritive value of the wheat protein.

The lack of significance among diets for both biological value and protein efficiency ratio indicates that irradiation up to 1,000,000 rep does not affect the availability to the animal organism nor the growth promoting value of the wheat protein.

The significantly higher nitrogen intake and urinary nitrogen and the significantly lower biological value during the second seven day period may indicate that the nitrogen intake during this period was higher than the amount needed for maximum growth and therefore partly catabolized and excreted, resulting in a lower biological value than the true one.

The lack of correlation between biological value and creatinine nitrogen percentage in the urine could be due to rapid changes in the creatinine excretion of the growing animals.

The results of the study presented appear to be encouraging for the application of irradiation to wheat preservation in dosages up to 1,000,000 rep.

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INTRODUCTION

Treatment with ionizing radiation has been proposed as a means of destroying grain-infesting insects and molds. There is evidence that certain chemical and physical properties of wheat protein are affected by such treatment.

Lloyd et al. (1956) found that the viscosity of gluten sols was lowered upon irradiation, while Milner and Yen (1956) observed a decreased loaf volume in bread baked from irradiated flour, with a decrease in gluten imbibitional capacity. Nicholas et al. (1958) likewise found a decrease in loaf volume for bread prepared from irradiated milled wheat and irradiated flour. In the case of wheat a significant decrease in volume appeared at a lower level of irradiation than for flour.

Such reports suggest an alteration of protein structure which may affect the availability of the protein to the animal organism. A knowledge of any change in biological value is necessary in evaluating the nutritive importance of food.

Johnson and Metta (1956) reported that irradiation lowered the biological value of pea and milk protein, while no change occurred in the biological value of lima bean and beef protein. It would appear that irradiation affects the availability of some food proteins and not others.

The study reported herein was undertaken to determine whether irradiation, at different levels, has any effect on the

availability, expressed as biological value, of wheat protein to the animal organism.

REVIEW OF LITERATURE

Types of Irradiation and Mode of Action

About a year after Roentgen discovered X-rays, Minck (1896) discussed the effect of Roentgen rays on bacteria and the possibility of their eventual therapeutic use. Since 1896, many investigators have studied the effect of irradiation on biological materials. However, only in the last fifteen years has considerable interest in the possibilities of food preservation by ionizing radiation been aroused.

Various types of ionizing radiation have been considered suitable for food preservation. However, as pointed out by Robinson (1954), more recent research has been concentrated on three types of irradiation, X-rays, cathode rays and fission waste products, made up of alpha, beta and gamma rays.

X-rays are among the oldest known artificially-produced radiations. When the accelerating voltage in the X-ray source is around 100 kilovolts or less, the generated rays are referred to as soft X-rays. On the other hand, if the accelerating voltage is about 185 kilovolts or more, the produced rays are called hard X-rays. The penetrating power of hard X-rays is relatively large which is an advantage in food preservation. However, the very low efficiency of generating these hard X-rays makes them uneconomical to use for large scale food preservation. A disadvantage of soft

X-rays is their relatively low penetrating power. This may be controlled by the design of X-ray units, but the over-all depth of treatment is limited by the high absorption of the soft X-rays (Robinson, 1954).

Cathode rays are artificially-produced electrons or beta particles. Their penetrating power into matter is less than that of X-rays of corresponding voltage, but still sufficient for use in food preservation. The efficiency of producing cathode rays is much higher than for generating hard X-rays. This makes cathode rays more economical for commercial food preservation.

Commercially, the most practical type of irradiation will probably be gamma rays of fission waste products. For all practical considerations, gamma rays are the same as X-rays, yet have much greater penetrating power. Considerable quantities of these radio active fission products have been produced as byproducts in the operation of nuclear reactors or atomic piles and have been stored in underground tanks. Not enough experience in the use of atomic waste products has been obtained to evaluate their importance completely.

The unit of power is the unit of ionization, namely the roentgen (r) in the case of X-rays and the roentgen-equivalent-physical (rep) in the case of cathode and gamma rays. This is equivalent to the absorption of 93 ergs per gram of material of unit density.

Two mechanisms have been proposed to explain the effect of ionizing radiation on several compounds. The "direct hit" or

target theory is based on investigations of biologists (Lea, 1940) and postulates that a swiftly moving charged particle, passing through complex biological material may alter or destroy the biological function of the complex.

Investigations of a later date, however, indicate that direct hits may be responsible for some specific radiation effects, but many of the effects are caused by the solvent. This theory is known as the "activated solvent" theory. In this case part of the energy absorbed by the solvent is transferred to the solute.

Dale et al. (1943) demonstrated that indirect action occurs when the short-lived ion disappears and gives rise to radicals and intermediate chemical compounds in the liquid medium. The decomposition of pure water upon irradiation is at present quite well understood, due to the work of Fricke (1935), Fricke and Hart (1935), Fricke et al. (1938), Weiss (1944, 1946) and Allen (1948). It is now generally believed that the activated water consists of free hydrogen atoms and hydroxyl radicals, formed by decomposition of water in the following manner:

$$H_2O \xrightarrow{\sim\sim} H_2O^+ + \text{electron}^-$$

$$\text{electron}^- + H_2O \longrightarrow H_2O^-$$

$$H_2O^+ \longrightarrow H^+ + OH$$

$$H_2O^- \longrightarrow OH^- + H$$

The hydroxyl radical is a strong oxidizing agent, while the hydrogen atom is a strong reducing agent. Any oxidizable solute is oxidized and any reducible solute reduced. The free radicals may also recombine and form oxygen, hydrogen, hydrogen peroxide or water, or

in the presence of oxygen, the very reactive HO_2 radical may be formed. These reactions prevent the free radicals from reacting with the solute. In the presence of organic material, no formation of hydrogen peroxide is observed, but the evolution of hydrogen is greater (Tobias, 1951). Both mechanisms may apply within the same system, although the indirect theory offers a wider basis for chemical changes (Ise and Fox, 1955).

Evidence for the activated solvent theory has been found in several studies investigating the influence of irradiation on aqueous solutions of amino acids. It was found that dilute solutions were relatively more affected by a given dose of radiation than those of higher concentration (Stenström and Lohmann, 1928; Bhatia and Proctor, 1951; Proctor and Bhatia, 1952). It is of interest to know that Goldblith and Proctor (1949) found that the same dilution phenomena occurred for carotene dissolved in ether. Presumably other compounds are formed by the irradiation of ether, acting in the same way as the hydroxyl radicals and hydrogen atoms of the activated water.

Observed Physical and Chemical Changes of Amino Acids Due to Irradiation

The first knowledge about the effect of irradiation on proteins was obtained mainly with amino acids, because of the complexity of the protein molecule.

Stenström and Lohman (1928) exposed tyrosine in weak aqueous solutions of different concentrations to Roentgen radiation. A

change of tyrosine in regard to the phenol group was observed. The change was directly proportional to the dose of radiation absorbed and varied only slightly with the concentration. With high dilutions only a small decrease in the efficiency of the irradiation was observed.

Allan et al. (1937) exposed solutions of amino acids, their derivatives and dipeptides to the action of cathode rays and ultraviolet light. Ammonia was liberated from all compounds upon irradiation but the yield from the dipeptides in which the hydroxyl of the carboxyl group was replaced by a NHC_6H_5 residue was generally less than from the corresponding amino acids in which the carboxyl was free.

Dale et al. (1949) measured the amount of ammonia derived from 0.13 M amino acid solutions after exposure to an X-ray dose of 166,000 r. The alpha amino acids glycine, arginine and alanine were deaminated to about the same extent, while the deamination of histidine exceeded the average value. This may be due to a contribution from the glyoxaline portion of the molecules and/or to a weakening of the strength of the carbon-nitrogen bond in the alpha amino group through the vicinity of the glyoxaline.

Bhatia and Proctor (1951) exposed solutions of histidine monohydrochloride in different concentrations to cathode-ray radiation from 100,000 up to 1,000,000 rep. They reported that histidine monohydrochloride decomposed upon irradiation due to deamination of the alpha amino group and fission of the imidazole ring. They found also that the decomposition over the range of concentrations

studied, was related exponentially to the dosage and dilute solutions were relatively more affected than concentrated ones.

Dale and Davies (1949) and Dale et al. (1949) found that when amino acids were irradiated with a large X-ray dose, the formation of ammonia increased with the concentration of the amino acid. The ionic yield for a glycine solution as well as for dry glycine rose to a value far above unity, while up to this time the ionic yields observed for biological material were never higher than unity. Ionic yield was defined as the number of molecules of ammonia liberated for every 32.5 electron volts of X-ray energy absorbed. In order to explain the high ionic yield obtained in the deamination of glycine solutions, the authors suggested two possible mechanisms.

The first mechanism is based on the suggestion of Weiss (1944) that the reaction of ionizing radiations is due to the formation of radicals produced by the splitting of water molecules. This should give an ionic yield of not more than unity if one hydroxyl radical is involved in the deamination of one mono-amino-mono-carboxylic acid and one effective radical pair is formed for each ion pair (Dale and Davies, 1949). However additional radicals may be formed by excitation or direct dissociation as well as by neutralization of charged ions as suggested by Dainton (1948) and Miller (1948). The latter suggested that in the case of his inorganic solutes, a total of about three pairs of radicals are formed per ion pair. If both radicals are effective in reacting with the solute, either directly or indirectly by the mechanism suggested by

Miller, then a much higher ionic yield could be obtained, without any form of a chain reaction.

The other alternative is a chain reaction, which is suggested as follows:

$$OH + {}^{+}HN_3.CH_2.COO^{-} \longrightarrow OH.CH_2.COO^{-} + NH_3^{+}$$

$$NH_3^{+} + H_2O \longrightarrow NH_4^{+} + OH$$

The reformed hydroxyl radical can then collide with another glycine molecule and so carry on the chain.

These mechanisms of course do not explain the high ammonia yield from dry glycine.

Proctor and Bhatia (1952) studied the effect of high voltage cathode rays in doses ranging from 100,000 - 1,000,000 rep on aqueous solutions of tryptophane, phenylalanine, tyrosine and cystine in various concentrations. Each of the amino acids was decomposed upon irradiation and the decomposition was related exponentially to the dose, while the dilute solutions were relatively more affected by a given dose than the more concentrated ones. The data indicated an indirect mechanism in which the action of high voltage cathode rays on these amino acids took place through free radicals being intermediates. The same authors (1953) conducted another study on the same amino acids with addition of histidine monohydrochloride monohydrate. The irradiation dosages ranged from 100,000 - 1,500,000 rep. As in the previous study it was found that the amino acids were deaminated upon irradiation with high voltage cathode rays. Histidine was deaminated to the greatest

extent and the order of deamination was: histidine - cystine - phenylalanine - tyrosine - tryptophane. Also spectroscopic evidence indicated that irradiation with high voltage cathode rays caused the fission of the ring structure of the aromatic amino acids.

Jayson, Scholes and Weiss (1954) likewise reported that the indole ring of tryptophane had been opened upon irradiation to form formyl-kynurenine. Under the conditions of the experiment the presence of oxygen seemed to be necessary.

Johnson, Scholes and Weiss (1951) observed that irradiation of alanine in the presence of oxygen produced pyruvic acid, while in vacuo acetaldehyde was formed. The authors suggested that the intermediates for the production of pyruvate and acetaldehyde were probably not the same.

Stein and Weiss (1948) reported that irradiation of serine by X-rays yielded besides ammonia also glycolaldehyde, while the presence of hydroxypyruvic acid was indicated.

Maxwell, Peterson, and Sharpless (1954) investigated the effect of the action of soft X-rays on oxygen-free aqueous solutions of glycine. The following products were identified as primary products arising from reactions induced by the X-ray radiation: ammonia, methylamine, glyoxalic acid, formaldehyde, acetic acid, formic acid, carbon dioxide and hydrogen. Hydrogen peroxide and carbondioxide were identified as secondary products. The authors proposed the following reactions to account for the observed products:

$$CH_2NH_2COOH \begin{cases} + X_1 \longrightarrow CH_3COOH + NH_3 \\ + X_2 \longrightarrow HCOCOOH + NH_3 \\ + X_3 \longrightarrow HCHO + NH_3 + CO_2 \\ + X_4 \longrightarrow HCOOH + NH_3 + CO_2 \end{cases}$$

These are indirect reactions, utilizing free radicals formed as the result of energy absorbed by the water, while the fifth reaction,

$$CH_2NH_2COOH + X_5 \longrightarrow CH_3NH_2 + CO_2$$

is a direct one, utilizing energy absorbed by the glycine.

Sharpless, Blair and Maxwell (1955) found that the radiation decomposition of alanine parallels in a remarkable degree that of glycine. Each product from glycine (except formic acid) had its analog in alanine.

Garrison and Weeks (1956) exposed aqueous solutions of glycine to radiation with cyclotron-produced helium ions. In addition to the products found by Maxwell, Peterson and Sharpless (1954) they identified also succinic acid, aminosuccinic acid and diaminosuccinic acid. The authors suggested that the decomposition of glycine at the locus of the carbon-nitrogen bond to give ammonia and glyoxalic acid occurs through the intermediate formation of iminoacetic acid. It is possible however that at a low radical concentration (as in the case of X-rays) the reactions may be different than in the case of cyclotron irradiation.

Sulfur in organic linkage as well as in its elemental form and in the form of thiosulfate is very sensitive to ionizing radiation. Dale and Davies (1951) observed hydrogen sulfide liberation

when aqueous solutions of cysteine hydrochloride and glutathione, in its reduced form, were exposed to radiation with X-rays. It was found that for a given concentration the amount of hydrogen sulfide was directly proportional to the dosage (up to 100,000 r) and independent of the oxygen content of the solution. The efficiency of the hydrogen sulfide production was in general less than that of ammonia formation from amino acids. It was also found that no ammonia was produced from cysteine at pH 2, 4 or 6, whereas the hydrogen sulfide production rose to a maximum at about pH 6.5. The radiation energy seemed to be used at least partly for hydrogen sulfide production, rather than for deamination. The same authors did not find hydrogen sulfide formation from cystine, while on the other hand, Proctor and Bhatia (1952) observed the liberation of hydrogen sulfide upon high voltage cathode ray irradiation of cystine and suggested the decomposition of cystine at the disulfide linkage. The different results obtained by Dale and Davies (1951) might be due to the lower irradiation dosages used by these investigators.

While the influence of ionizing radiations on amino acids are fairly well known, it seems that the physical and chemical changes due to the irradiation of intact protein are not as well understood.

Observed Physical and Chemical Changes of Proteins Due to Irradiation

The denaturation of the protein molecule was one of the first effects of irradiation upon protein observed.

Arnow (1935) found that irradiation of egg albumin solutions with alpha particles resulted in the formation of a visible coagulum if the initial pH of the solution was that of the isoelectric point. Changes in the ultraviolet absorption spectrum were observed, namely an increased absorption of the solutions at or below the isoelectric point and a decrease for solutions above the isoelectric point.

Clark (1935) studied the denaturation of isoelectric egg albumin by ultraviolet radiation and found that three distinct processes were involved. First occurred a light denaturation of the albumin molecule, followed by a reaction between the denatured molecule and water, which may be similar to heat denaturation but occurred at a low temperature. The last part of this process consisted of the flocculation of the denatured molecule to form a coagulum.

Barron and Finkelstein (1952) stated that the changes in the absorption spectrum produced in proteins (serum albumin, gamma globulin and egg albumin) by X-irradiation were different from the changes observed on irradiation with ultraviolet light or denaturation by heat or treatment with acids or alkalies. They were however similar to changes in the absorption spectrum of X-irradiated tyrosine. The absorption spectrum changes seemed to be due to oxidation of tyrosine residues and other oxidizable groups.

They found, contrary to Arnow (1935), larger increases of light absorption upon irradiation of alkaline solutions.

Irradiation of 0.07% aqueous solutions of serumalbumin with 75,000 r. at 25° C. produced precipitation, which did not occur on irradiation at the temperature of ice water. Precipitation was also avoided by increasing the protein concentration or by the addition of salts, as NaCl, NaBr, $NaNO_3$, and NaSCN.

The same authors compared the effect of irradiation on solutions of serum albumin in the absence and presence of oxygen and found a greater increase for oxygenated solutions at wave lengths around 2400 A. They concluded that the action of ionizing radiation on serum albumin solutions was in part indirect and that the O_2H radicals, one of the products of oxygenated water irradiation, contributed their share to the observed effect, since the absence of dissolved oxygen would have no influence on the efficiency of direct collisions. It was shown earlier (Barron et al., 1952) that hydrogen peroxide, the other product of irradiation of oxygenated water, played a negligible role in the action of ionizing radiations.

Viscosity changes have also been used to evaluate alterations produced by irradiation of proteins. Arnow (1935) showed that the viscosity of egg albumin solutions exposed to alpha particles was raised at or below the isoelectric point of egg albumin, but lowered if the pH was above the isoelectric point. Barron and Finkelstein (1952) found a small increase in viscosity on X-irradiation of serum albumin and globulin, most marked on irradiation in alkaline solution and suggested an increased asymmetry of the molecule. Scheraga and Nims (1952) observed an increased

viscosity of fibrinogen solutions of pH 6.5 upon X-irradiation (up to 300,000 r), while at an irradiation level of 600,000 r. gel-formation occurred. Ultracentrifugal data indicated that polymerization processes were involved, but also fragmentation occurred.

Splitting of hemocyanin, hemoglobin and serum albumin molecules was also found by Svedberg and Brohult (1939).

Lloyd et al. (1957) treated dry gluten and gluten sols with X-radiation dosages up to 700,000 r. Viscosities were determined at 30° C. and pH 3.2. It was found that the viscosity of the sols made up from the irradiated dry gluten decreased linearly with increasing dosage, whereas the decrease in viscosity of the irradiated sols was greater and nonlinear. The authors suggested that the decrease in viscosity indicated that the protein molecules were broken down into shorter and/or more symmetrical particles, while the greater decrease in viscosity of the irradiated sols pointed toward a more efficient mechanism responsible for the degradation of the protein molecules in solution than in the dry gluten form. The influence of temperature was insignificant, and dissolved oxygen did not affect the obtained results. It was also found that dilute gluten sols were relatively more affected than the more concentrated ones. This might indicate an indirect mechanism for the action of X-rays on gluten sols.

Jayko and Garrison (1956) proposed a mechanism for the radiation-induced cleavage of the peptide chain. The studies were carried out with cyclotron irradiation of diethylamine solutions at pH 3 in the presence of oxygen. The following reactions, due

to indirect action of radiation were suggested:

$$R\text{-}CO\text{-}NH\text{-}CH_2\text{-}R + OH \longrightarrow R\text{-}CO\text{-}NH\text{-}CH\text{-}R$$

$$R\text{-}CO\text{-}NH\text{-}CH\text{-}R + O_2 \longrightarrow R\text{-}CO\text{-}N{=}CH\text{-}R + HO_2$$

$$R\text{-}CO\text{-}N{=}CH\text{-}R + H_2O \longrightarrow R\text{-}CO\text{-}NH_2 + R\text{-}CHO$$

In the action of ionizing radiation on foods the protective action of certain compounds against irradiation influences may play an important role. Proctor and Goldblith (1948) studied the effect of hard X-rays and cathode rays on pure solutions of niacin to which certain compounds were added. In the case of X-rays, no protection was found from methionine and ascorbic acid but ascorbic acid itself was protected by niacin. When cathode rays were used methionine showed a protective action for niacin, while glycine, cystine and cysteine did not have any effect.

Feinstein (1951) observed that cysteine added to an alkaline nucleoprotein solution before X-radiation prevented largely the expected reduction in viscosity. Cysteine added after X-radiation was much less effective in this respect.

Proctor and Goldblith (1952) and Proctor et al (1952) demonstrated that ascorbic acid, D-isoascorbic acid, sodium D-isoascorbate and niacin had a protective action against the influence of cathode ray radiation when combined with pepsin, histidine monohydrochloride, suspensions of red blood cells and samples of chopped cured meat products. The authors suggested that the protective agents were acting as free radical acceptors in competing with the other compounds of the system for the free radicals formed as primary products of the irradiation of water.

Scheraga and Nims (1952) found that thiourea, cysteine and glutathione showed a protective action when added to fibrinogen solutions before X-radiation. The formation of heavier compounds, as found on irradiation, was decreased or eliminated altogether.

On the other hand Lloyd et al. (1956) in their study on gluten sols, did not find any protective effect from the addition of cysteine hydrochloride, glutathione and 2-mercaptoethanol.

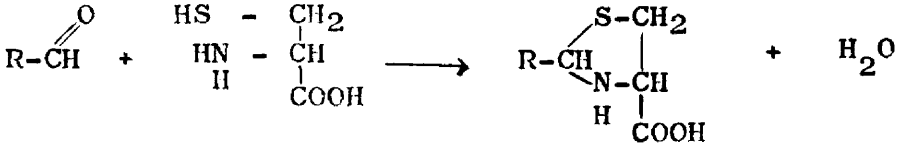
Eldjarn et al. (1955), using sulfur compounds labelled with S^{35} demonstrated that the protective action of cysteamine and cystamine ($HS-CH_2-CH_2NH_2$ and $NH_2CH_2-CH_2-S-S-CH_2-CH_2NH_2$) was due not so much to their action as free radical acceptors, but rather to a specific chemical reaction of cysteamine with free sulfhydryl groups of the protein molecule in which cystamine forms a disulfide linkage, according to the scheme:

$$\text{Protein-S-H} + \underset{\text{cystamine}}{\text{R-S-S-R}} \longrightarrow \text{Protein-S-S-R} + \underset{\text{cysteamine}}{\text{H-S-R}}$$

The resulting disulfide linkage appears to be much more resistant to the action of ionizing radiation than the unprotected sulfhydryl group, which is extremely radiosensitive. The blocking of the sulfhydryl group is temporary.

Littman et al. (1957) studied methods to prevent hydrogen sulfide and mercaptan formation, both contributors to the odor problem in food irradiation. It was found that the masking of the sulfhydryl groups, especially in cysteine and glutathione, by specific chemical reactions, was much more effective than protecting them by competing reactions. Work done by Schubert (1935, 1936) showed that a number of carbonyl compounds were capable of reacting with sulfhydryl

compounds forming mercaptals, with loss of the characteristic sulfhydryl reactions, particularly in the case of alpha amino groups, since stable thiazole rings could be formed:



Littman et al. (1957) tried a number of these carbonyl compounds and found that cysteine was best protected by glyoxal, formaldehyde, pyruvic acid, pyruvic aldehyde, diacetyl and glyceraldehyde, which reduced the amount of hydrogen sulfide formed to 10-15% of that found in unprotected solutions. Glutathione was best protected by glyoxal, pyruvic acid and formaldehyde. Ascorbic acid on the other hand had only a very slight protective action on both cysteine and glutathione.

Proteins in their natural environment seem to be much more stable against the influence of ionizing radiation than pure proteins or amino acids. Proctor and Bhatia (1950) suggested that this might be due either to the protective action of other compounds present or to the concentration in which they are present in the food.

Proctor and Goldblith (1951) irradiated ground beef patties and protein hydrolysates with cathode rays at 1,500,000 rep and found that the losses in essential amino acids were small.

Proctor and Bhatia (1950) studied the effect of high voltage cathode rays, in dosages ranging from 900,000 - 5,700,000 rep, on haddock fillets. The amino acid destruction of arginine,

histidine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane, valine and cystine was determined. No significant destruction of any of these ten amino acids was found.

Brownell et al. (1955) found that loaves of bread baked from flour irradiated with gamma rays at 500,000 - 1,000,000 rep showed a smaller volume than the control. The first slight flavor changes could be detected at an irradiation level of 50,000 rep.

Yen et al. (1956) irradiated intact dry and damp grain with gamma rays and found that dosages in excess of 625,000 rep produced an increased fluorescence, which is an indication of sugar-protein interaction (non-enzymatic browning), while a decrease in protein solubility generally followed fluorescence changes. Both changes occurred particularly after a period of storage and were the same for dry (12% moisture) and damp (20% moisture) grain.

Milner and Yen (1956) continued the above mentioned study and treated dry wheat with gamma rays at levels up to 1,000,000 rep. The results showed that irradiation levels beyond 250,000 rep produced decreased loaf volume in bread baked from this flour, while the imbibitional capacity of the gluten decreased with increasing dosage.

Nicholas et al. (1958) treated wheat and wheat flour with cathode rays. It was found that loaf volumes were not significantly different among treatments up to 500,000 rep in the case of bread made from irradiated flour. In the case of bread prepared from the milled irradiated wheat however, dose levels of 50,000 rep

gave significantly higher volumes than at 250,000 rep. The first flavor changes were detectable at about 50,000 rep.

These studies suggest a change in protein structure, which may in some cases alter the availability of the protein to the animal organism.

Effect of Ionizing Radiation on the Nutritive Value of Foods

Growth and reproduction of experimental animals fed irradiated diets, together with biological value of proteins have been used as a means of evaluating the nutritive value of foods treated with ionizing radiations.

Luckey et al. (1955) fed a high vitamin and semi-synthetic diet to a number of three generations of mice after (a) no treatment, (b) steam sterilization, and (c) 2,800,000 rep cathode ray radiation. General appearance, growth and reproduction were similar in all three groups of mice.

Poling et al. (1955) compared raw ground beef, irradiated with cathode rays at a level of 2,000,000 rep with similar unirradiated beef by feeding experiments with rats through three generations. Two-thirds of the diet fed was composed of meat. The small and occasionally statistically significant decreases in growth, food efficiency, and reproduction found in the group of animals on the diet containing irradiated meat, were considered by the authors to be due to slightly decreased nutritional quality similar to that which occurs during heat sterilization.

Brownell et al. (1956) conducted a feeding experiment in which wheat, irradiated with gamma rays at a level of 10,000 rep, was fed as 70% of the diet to rats. It was found that the groups on the irradiated diet grew slightly better than did the controls, even though their starting weight was inferior.

Burns et al. (1956) irradiated nutritionally complete poultry mashes with 3,000,000 rep gamma rays and fed these to chickens. The diet was supplemented with vitamins after irradiation. A slightly reduced rate of growth and a slightly delayed maximization of hatchability of eggs was found for the pullets on the irradiated diet. The authors however suggest that the lower body weight may reflect mainly a difference in fat deposition of the experimental animals and that the delayed hatchability be a consequence of this.

Teply and Kline (1956) tested twenty-four food items irradiated with 3 and 6 million rep gamma rays in short term rat feeding experiments (8 weeks). The foods were mixed with a complete semi-synthetic basal ration at a 35% level on a dry weight basis. The items tested were: apricots, asparagus, Brussels sprouts, cabbage, cauliflower, celery, cherries, chicken, corned beef, cranberries, crackers, gelatin dessert powder, macaroni, mushrooms, nutrolls, pears, peas, pork sausage, sweet potatoes, white potatoes, cake, salmon, shrimp and tuna. In most cases no significant difference in growth on control and irradiated diets were found. Radiation of cauliflower, celery and white potatoes however produced a moderate increase in growth rate, possibly due to an increase in

acceptability or digestibility, because they were treated in the raw state. The irradiation of apricots and chicken produced a slight decrease in growth rate, while irradiation of the gelatin dessert powder caused a definite lowering of growth rate of approximately 10%. Microbiological assays showed destruction of several amino acids, however feeding studies with sucrose and gelatin fed separately indicated that the sucrose component was responsible for most of the growth effect. A definite lowering of nutritive value of gelatin for the chick with destruction of arginine and glycine upon irradiation was also reported by Richardson (1956). Similar to the experiment of Teply and Kline (1956), Kraybill et al. (1956) investigated twelve other food items: ground beef, fresh ham, sliced bacon, haddock, green beans, corn, beets, frozen strawberries, peaches, bread, cereal, and powdered whole milk. Excellent growth and food consumption were obtained on all diets at the two irradiation levels.

Bubl and Butts (1956) found that the irradiation of organ meats, fed to rats had no effect on the growth of the animals. The breeding performance of the females showed also a comparable pattern with the control group.

Johnson and Metta (1956) and Metta and Johnson (1956) studied the effect of 3 million rep gamma irradiation on digestibility and biological value (using the Thomas-Mitchell method) of pea, lima bean, beef and milk protein. Digestibility and biological value of pea and milk protein was lowered by approximately 8%. The digestibility remained the same. The addition of cystine to milk

brought the biological value to a level above that of the raw milk, while histidine and lysine showed no supplementing effect, indicating that the effect of radiation of milk was due to the alteration of cystine, which became the limiting amino acid. This may be due to destruction or binding of cystine.

Read et al. (1958) treated fourteen food items with gamma-irradiation in dosages of 3,000,000 and 6,000,000 rep. The foods used were: ground beef, fresh ham, haddock fillet, turkey, bacon, whole kernel corn, leaf spinach, beets, green snap beans, peaches, strawberries, canned bread, military cereal bar and whole powdered milk. The food items tested were fed ad libitum to rats for eight to twelve weeks as 35% of the dry weight of the diet. The remaining 65% of the diet consisted of a non-irradiated nutritionally adequate semi-synthetic ration. Weight gain was used as an index of toxicity, assuming that the nutritional needs of the experimental animals had been satisfied. Thirteen foods were found to be non-toxic by this technique. A suggestion of low level toxicity of 6,000,000 rep irradiated peaches was obtained. It seems questionable whether in this study toxicity rather than nutritional adequacy was tested. With 35% of the diet consisting of the experimental foods, it would appear that the nutritional adequacy of the foods tested, might influence the weight gain of the animals.

In the light of the reported changes in protein structure, such as viscosity changes, hydrogen sulfide formation, cleavage of the peptide chain with aldehyde formation and the lowered biological

value found for pea and milk protein upon irradiation, it seems of considerable interest to investigate the possible changes in nutritive value of wheat protein at different levels of irradiation, especially since no work of this nature has been reported for this food item.

EXPERIMENTAL PROCEDURE

Diets

Four lots of ground wheat were treated with high voltage cathode ray ionizing radiation in the following doses:

50,000 rep

100,000 rep

500,000 rep

1,000,000 rep

A fifth lot of wheat was not irradiated and was used as a control. The five wheat samples were supplemented with vitamins, minerals and corn oil so that the percentage composition of the five diets was exactly the same (Table 1). The only variable was the dosage of radiation to which the wheat samples were exposed.

The lowest radiation dosage, 50,000 rep, was the level at which the first flavor changes could be detected in cake and bread baked from irradiated flour (Brownell et al., 1955) and in bread made from milled wheat and wheat flour treated with ionizing radiation (Nicholas et al., 1958). The upper level of irradiation was chosen somewhat above the dosage of 750,000 rep needed to destroy all fungal growth. The inactivation of insects takes place at a lower level, while most enzymes are destroyed at much higher levels. Destruction of enzymes however does not seem to be necessary for effective preservation of wheat (Hasset and Jenkings, 1952; Yen et al., 1956; Pommerantz, 1956 and Milner, 1957).

TABLE 1

COMPOSITION OF THE EXPERIMENTAL DIETS

Ingredients	Percentage
Wheat	90.4
Corn oil	5.0
Wesson's mineral mixture[1]	4.0
Vitamin mixture[2]	0.6

[1]Wesson (1932)

[2]The vitamin mixture provided per kilogram of diet:

2 mgm thiamine hydrochloride, 3 mgm riboflavin, 3 mgm pyridoxin hydrochloride, 20 mgm nicotinic acid, 10 mgm calcium pantothenate, 0.08 mgm folic acid, 800 mgm choline, 170 I. U. vitamin A and 3000 I. U. vitamin D.

Experimental Animals

Forty young adult male albino rats of the Sprague-Dawley strain, weighing about 200 gms, were divided into five groups, each consisting of eight animals. They were housed in individual metabolism cages and food and water were allowed ad libitum. Each group received one of the wheat diets. A period of seven days, allowed for adjustment to the diet, was followed by two collection periods, each of seven days, during which urine and feces were collected. Food intake and body weight were recorded at the beginning and end of each of the two collection periods. Feces, kept separate from the urine by fine wire mesh, were removed from the cage once a day, pooled for the entire seven days, dried for 24 hours in an oven at 50° C. and ground to a fine powder. The wire meshes and pyramidal-formed bottoms of the cages were sprayed with hot 2% boric acid to prevent nitrogen losses from the urine. In the middle and at the end of each collection period, the wire mesh and bottom of each cage were washed down with 2% boric acid. Urine and washings were filtered (filter paper Whatman no. 4) into a collection flask containing 10 cc 0.5% sulfuric acid. The filter paper was saved and air dried. Any spilled food collected on the filter paper was washed with water to remove traces of urine, dried, weighed and subtracted from the recorded food intake. Urine and washings from cages and food were pooled for each collection period, preserved with toluene and kept under refrigeration.

Nitrogen determinations of food samples, urine, feces and filter paper were made by the boric acid modification of the Kjeldahl-Gunning method (A.O.A.C., 1955).

Evaluation of the Nutritive Value of the Protein

The protein efficiency ratio, expressed as gain in body weight per gram of protein eaten, was determined for each of the five diets for both collection periods.

The biological value was expressed as the percentage of the total intake stored, according to the formula:

$$\text{Biological value} = \frac{\text{N intake} - (\text{fecal N} + \text{urinary N})}{\text{N intake}} \times 100$$

as well as on the basis of digested protein by the following formula:

$$\text{Biological value} = \frac{\text{N intake} - (\text{fecal N} + \text{urinary N})}{\text{N intake} - \text{fecal N}} \times 100$$

The two formulas measure the biological value of protein for growth purposes only. The Thomas-Mitchell method on the other hand takes accound of maintenance as well by considering the metabolic and endogenous losses separately from the total fecal and urinary excretion, metabolic and endogenous nitrogen being determined on a nitrogen-free diet or on a diet containing a small amount of egg protein. (Mitchell, 1924, 1944; Maynard and Loosli, 1956)

It seems questionable whether nitrogen excretion during a protein-free period measures the metabolic fecal nitrogen under conditions of protein feeding. Barnes and Bosshardt (1946), in studies on mice, found that there was a regular progression in

fecal nitrogen as the protein content of the diet increased. However with a very low protein diet, there was a marked deviation in fecal nitrogen from the regular progression. This may influence considerably the calculation of the biological value by the Thomas-Mitchell formula.

The validity of the endogenous nitrogen measurement in the determination of the biological value of a protein has also been questioned. Allison et al. (1946) found that the biological value of proteins was markedly increased when fed to dogs in a state of hypoproteinemia. As in small animals, such as the rat, very short periods of protein-free diets may result in a considerable depletion of the body stores of protein, this may alter the calculation of the biological value. Small amounts of egg protein incorporated in the diet may help to avoid protein depletion, however, this protein may not be completely absorbed (Barnes and Bosshardt, 1946; Albanese, 1950).

Considering the factors of uncertainty involved in the calculation of the biological value of protein by the Thomas-Mitchell method, it seemed for the purpose of this study in which the main object was a comparison of the different biological values rather than obtaining absolute values, more desirable to use either of the two formulas mentioned above. Furthermore it seemed advisable to charge metabolic and endogenous losses against the protein being ingested at the time of the measurement.

The nutritive value of the wheat protein was also expressed by the creatinine nitrogen percentage in the urine, determined by

the Clark-Thompson procedure (1953). Murlin et al. (1948, 1953) found a high correlation between the biological value as expressed by the Thomas-Mitchell formula and the creatinine nitrogen percentage in the urine of human subjects and of dogs during the last days of any given period of protein ingestion. Hence it was considered of interest to investigate whether the correlation also existed in this study.

RESULTS

Nitrogen Balance

The nitrogen content of each of the five diets was found to be 2.4%, which is equivalent to 14% protein, using the factor 5.83, recommended for whole kernel wheat (F.A.O., February, 1947). The nitrogen intake of each rat for both seven day collection periods was calculated from the amount of food consumed. Data for the average nitrogen intake in grams for each group of animals for both periods are presented in Table 2.[1] The five diets seemed to be equally acceptable and no food refusals were noticed in any of the groups. No significant differences among the nitrogen intake of the rats on the five different diets were found. The intake during the second collection period however, was significantly higher (1% level) than that in the first period.[2]

Average values for the urinary nitrogen, fecal nitrogen and nitrogen balance, calculated per group of animals for each collection period are also included in Table 2. The nitrogen balance was found to be positive for all animals. No significant differences were found among the values for the different diets. The urinary nitrogen during the second seven day period however, was significantly

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[1]Complete tables with data for individual rats of the nitrogen balance data will be found in the Appendix, Tables 5, 6, 7 and 8.

[2]Tables of the results of the statistical analysis are presented in the Appendix, Table 13.

TABLE 2

AVERAGE NITROGEN INTAKE, URINARY NITROGEN, FECAL NITROGEN AND NITROGEN BALANCE IN GRAMS

Diet	Nitrogen Intake		Urinary Nitrogen		Fecal Nitrogen		Nitrogen Balance	
	Period I	Period II	Period I	Period II	Period I	Period II	Period I	Period II
Control	2.90	3.02	1.28	1.48	0.57	0.56	+1.05	+0.99
50,000 rep	2.83	3.10	1.17	1.44	0.55	0.58	+1.11	+1.08
100.000 rep	2.72	3.04	1.22	1.45	0.51	0.56	+0.98	+1.03
500,000 rep	2.80	2.83	1.33	1.42	0.49	0.51	+0.98	+0.90
1,000,000 rep	2.54	2.78	1.09	1.32	0.52	0.51	+0.92	+0.95

higher than that in the first period. For fecal nitrogen and nitrogen balance no significant differences between periods were found.

Protein Efficiency Ratio, Biological Value and Creatinine Nitrogen Percentage in the Urine

In Table 3 and Figure 1 are shown the average protein efficiency ratios, expressed as gain in body weight in grams per gram of protein consumed, for the rats fed the different diets during the two collection periods, each of seven days duration.[3] Statistical analysis showed no significant differences for the protein efficiency ratios among groups or between periods.

Biological values of the five diets, expressed as the percentage of the total nitrogen intake stored, according to the formula:

$$\frac{\text{nitrogen intake - nitrogen output}}{\text{nitrogen intake}} \times 100$$

as well as on the basis of digested protein by the following formula:

$$\frac{\text{nitrogen intake - nitrogen output}}{\text{nitrogen intake - fecal nitrogen}} \times 100$$

for all groups during both collection periods are presented in Table 4 . The average creatinine nitrogen percentages in the urine per group for both collection periods are also shown in Table 4. The biological values during the second seven day period were found

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[3]Complete tables with data for individual rats of protein efficiency ratios, biological values and creatinine nitrogen percentages in the urine will be found in the Appendix, Tables 9, 10, 11, and 12.

TABLE 3

AVERAGE PROTEIN EFFICIENCY RATIOS, EXPRESSED AS WEIGHT GAIN IN GRAMS PER GRAM OF PROTEIN CONSUMED

Diet	Period I	Period II	Both Periods
Control	1.12	0.78	0.95
50,000 rep	1.14	1.10	1.12
100,000 rep	1.04	0.99	1.01
500,000 rep	1.14	0.97	1.06
1,000,000 rep	0.75	0.96	0.86

FIGURE 1

AVERAGE PROTEIN EFFICIENCY RATIOS,
EXPRESSED AS WEIGHT GAIN IN GRAMS
PER GRAM OF PROTEIN CONSUMED

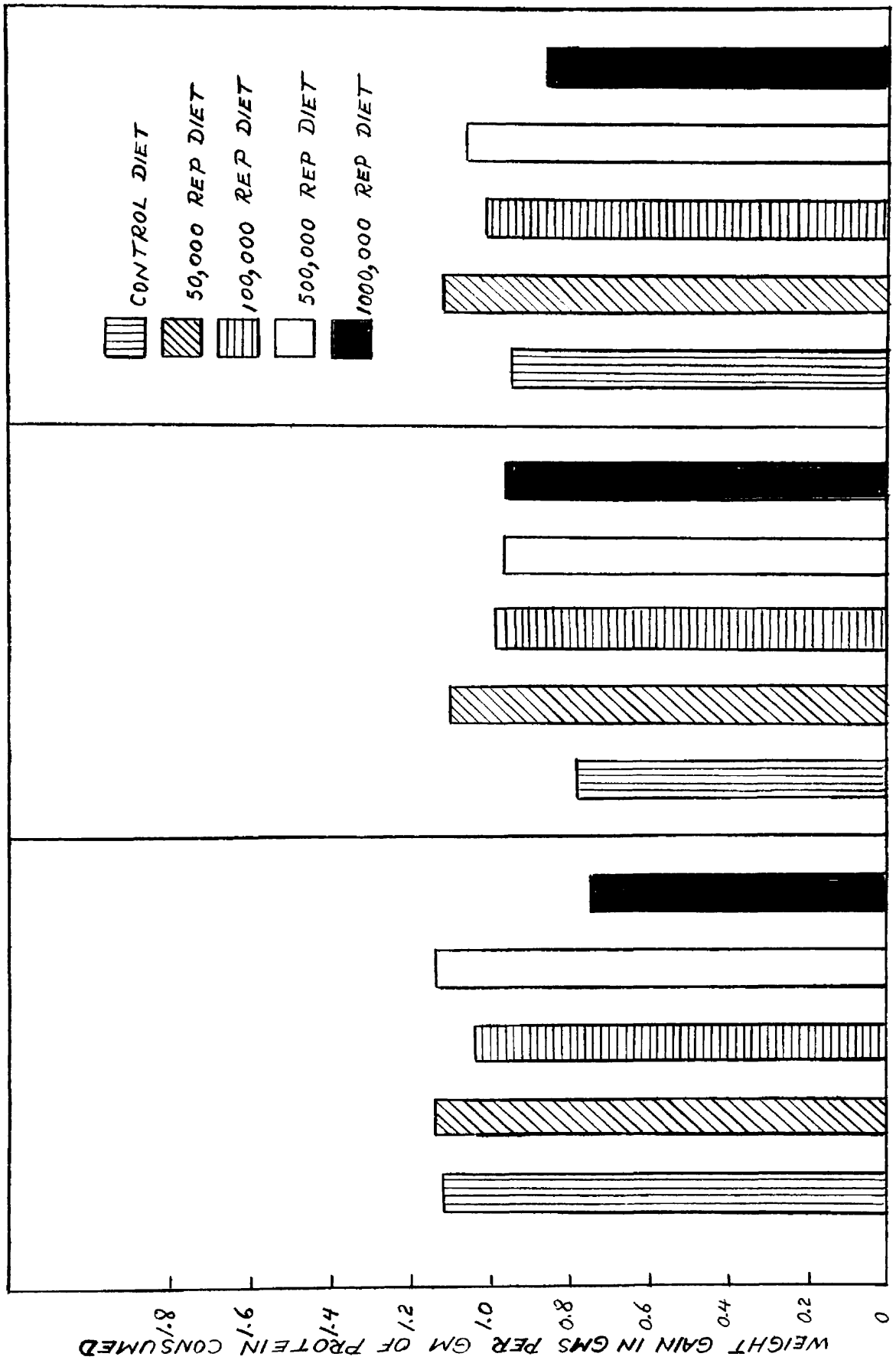
WEIGHT GAIN IN GMS PER GM OF PROTEIN CONSUMED
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
CONTROL DIET
50,000 REP DIET
100,000 REP DIET
500,000 REP DIET
1000,000 REP DIET
PERIOD I
PERIOD II
BOTH PERIODS

TABLE 4

AVERAGE BIOLOGICAL VALUES, EXPRESSED IN TWO DIFFERENT WAYS AND CREATININE NITROGEN PERCENTAGE IN THE URINE

Diet	A Biological Value Expressed as $\frac{\text{N intake - N output}}{\text{N intake}} \times 100$		B Biological Value Expressed as $\frac{\text{N intake - N output}}{\text{N intake - fecal N}} \times 100$		C Creatinine Nitrogen Percentage in the Urine	
	Period I	Period II	Period I	Period II	Period I	Period II
Control	36	33	45	40	4.33	4.23
50,000 rep	39	35	49	43	4.14	4.11
100,000 rep	36	34	44	42	3.86**	3.97**
500,000 rep	35	32	43	39	4.00	4.25
1,000,000 rep	36	34	45	42	4.44	4.24

**Significant at the 5% level.
Difference required for significance = $Q.s_{\bar{x}}$ = 0.40. (Snedecor, 1956)

to be significantly lower (5% level) than the first seven day period when they were expressed as the percentage of the total intake stored (Table 4, column A). They were also significantly lower (1% level) when expressed on the basis of digested protein (Table 4, column B). No significant differences were found among the five diets. On the other hand statistical analysis of the creatinine nitrogen percentage of the urine revealed that the differences among the diets were significant, the 100,000 rep diet being significantly lower than the 1,000,000 rep diet. Between the two periods, differences were not found to be significant.

There was no significant correlation between the creatinine nitrogen percentage in the urine and the biological values (expressed in the two different ways mentioned) nor between the creatinine nitrogen percentage and the protein efficiency ratio (Appendix Table 14).

DISCUSSION

Changes in protein solubility and viscosity, and gluten imbibitional capacity upon irradiation of wheat and wheat flour, even at levels below 1,000,000 rep, reported in the literature (Brownell et al., 1955; Lloyd et al., 1956; Yen et al., 1956; Milner and Yen, 1956 and Nicholas et al., 1958), suggest a change in protein structure. The results of the study reported herein however, indicate that structural changes resulting from these radiation levels, are not great enough to affect measurably the availability of the protein to the animal organism. These findings appear to be encouraging for the application of irradiation to wheat preservation in dosages up to 1,000,000 rep.

The nitrogen content of the five diets was found to be the same. According to Metta and Johnson (1956), this might indicate that irradiation caused little or no deamination of the amino acids of the intact protein.

The higher nitrogen intake during the second, seven day collection period could be due to the fact that the experimental animals were still growing. The positive nitrogen balance found for all animals during both collection periods is an evidence for this conclusion. On the other hand it might be possible that the lower food intake during the first period was the result of a relatively short time of adjustment to the diet. The latter

explanation however, seems unlikely since most investigators in studies on nitrogen metabolism and related subjects used successfully a period of adjustment of four to seven days with adult as well as with growing rats (Bricker and Mitchell, 1947; Mitchell et al., 1949 and Sibbald et al., 1956).

The protein efficiency ratio, expressed as gain in body weight per gram of protein consumed, was one of the ways in which the nutritive value of the wheat protein was evaluated. The limitations of this method have been critically reviewed by Mitchell (1944), indicating the error involved under conditions of ad libitum feeding, and that a gain in body weight might not be a true index for the increase in body protein. Barnes and Bosshardt (1946) on the other hand, believed that the maximal protein efficiency could not be obtained by paired feeding and showed that total body weight provided a good index for body protein if measurements were made at the level of protein intake providing maximal protein utilization. Both authors agreed however that the evaluation of a protein by this method might be useful in preliminary rating of proteins, but should not be used alone. Therefore in this study the protein efficiency ratio was considered as a useful addition to the other methods in evaluating the nutritive value of the wheat protein.

The lack of significance for the protein efficiency ratios among diets, indicated that radiation levels up to 1,000,000 rep did not affect the growth-promoting value of the wheat protein.

Statistical analysis of the biological values (Appendix Table 13) did not indicate in any way a difference in nutritive

value of the protein of the wheat exposed to different levels of irradiation. The lower biological values found during the second collection period may be explained by examining the formulas according to which the biological values were computed. In the first formula

$$\left(\frac{\text{nitrogen intake - nitrogen output}}{\text{nitrogen intake}} \times 100\right.$$

or

$$\left.\frac{\text{nitrogen balance}}{\text{nitrogen intake}} \times 100\right),$$

no significant differences were found for the numerator between both collection periods. The values for the denominator (nitrogen intake) on the other hand, were significantly higher during the second period, hence the biological value was expected to be lower during this period. In the second formula

$$\left(\frac{\text{nitrogen intake - nitrogen output}}{\text{nitrogen intake - fecal nitrogen}} \times 100\right.$$

or

$$\left.\frac{\text{nitrogen balance}}{\text{nitrogen intake - fecal nitrogen}} \times 100\right)$$

values for fecal nitrogen were also used and for these values no significant differences were found between periods. It follows that likewise in this case lower biological values could be expected for the second collection period. It might be possible that the higher protein intake during the second period was in excess of the amount needed to cause maximum growth, was partly catabolized and excreted, and thus gave a lower biological value than the true one.

The values for the creatinine nitrogen percentage in the urine do not seem to contribute much to the interpretation of the experimental results. No explanation can be found for the significantly low creatinine nitrogen percentage in the urine of the animals fed wheat irradiated at 100,000 rep. The total lack of correlation between the creatinine nitrogen percentages in the urine and the biological values and between the creatinine nitrogen percentages in the urine and the protein efficiency ratios makes it unlikely that in this study biological value parallels the creatinine nitrogen percentage in the urine. This is in contrast to the findings of Murlin et al. (1948, 1953). These authors observed a high degree of correlation between biological value and creatinine nitrogen percentage in the urine of the last days of any given period of protein ingestion and hence considered this constituent of urinary nitrogen as a base of reference for the evaluation of proteins. It might be possible that the creatinine in the urine of young growing animals is not as constant a value for each individual as in adult rats and that the correlation will not be found in growing animals. This is probably due to rapid changes in body weight, affecting the amount of creatinine excreted. Another factor may be the different way in which the biological value was expressed. The observations of Murlin et al. (1948, 1953) were made on adult human subjects and adult dogs and furthermore these investigators expressed the biological value according to the Thomas-Mitchell formula in which endogenous urinary nitrogen is subtracted from the total urinary nitrogen. The values

found for the creatinine nitrogen percentage in the urine in the present study may also be affected by other unknown circumstances, which makes their use in the evaluation of the nutritive value of the protein undesirable.

SUMMARY AND CONCLUSIONS

Four lots of ground wheat were exposed to high voltage cathode ray ionizing radiation in dosages ranging from 50,000 to 1,000,000 rep. A fifth lot was not irradiated and used as a control. Each of the five wheat samples was supplemented with vitamins and minerals and fed to eight young adult male albino rats in order to determine the nutritive value of the wheat protein. Feed and water were given ad libitum. Seven days were allowed for adjustment to the diet, followed by two succeeding periods, each of seven days, during which urine and feces collections were made. Food intake and changes in body weight were recorded for each seven day period. Total nitrogen of food and excreta and creatinine in the urine were determined for each collection period. The nutritive value of the protein was evaluated by means of protein efficiency ratio, biological value, expressed in two different ways, and creatinine nitrogen percentage in the urine.

No significant differences among diets were found for the biological values (expressed in both ways) or for the protein efficiency ratios. This indicates that irradiation up to 1,000,000 rep does not affect the availability to the animal organism, nor the growth promoting value of the wheat protein.

The nitrogen intake during the second collection period was found to be significantly higher and the biological values

significantly lower, than in the first period. This may indicate that the nitrogen intake during the second collection period was higher than the amount needed for maximum growth and therefore partly catabolized and excreted, resulting in a lower biological value than the true one.

No correlation could be found between biological value and creatinine nitrogen percentage in the urine. This was possibly due to a rapid changing creatinine excretion in the growing experimental animals or to other unknown factors affecting the creatinine excretion.

It would appear that the results of the study presented, are encouraging for the use of cathode ray ionizing radiation, in dosages up to 1,000,000 rep, for wheat preservation.

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APPENDIX

TABLE 5

NITROGEN INTAKE IN GRAMS

Rat No.	Diet									
	Control		50,000 rep		100,000 rep		500,000 rep		1,000,000 rep	
	Period I	Period II	Period I	Period II	Period I	Period II	Period I	Period II	Period I	Period II
1	2.57	2.79	2.78	2.97	2.53	2.92	2.80	2.88	2.08	2.44
2	2.56	2.94	2.83	3.61	2.64	2.75	2.49	2.73	1.59	2.30
3	3.12	2.89	3.10	3.32	2.73	3.70	2.68	3.17	2.56	2.84
4	3.38	3.66	2.92	3.16	3.29	3.22	3.02	3.08	2.57	2.13
5	2.95	3.03	2.55	2.90	2.97	3.24	3.48	2.71	2.85	3.00
6	2.87	2.86	2.48	2.58	2.52	2.68	2.73	2.59	2.81	3.40
7	2.70	2.69	2.87	3.07	2.39	2.84	2.58	2.65	3.13	3.11
8	3.07	3.31	3.10	3.21	2.66	2.97	2.66	2.84	2.71	2.99
Mean	2.90	3.02	2.83	3.10	2.72	3.04	2.80	2.83	2.54	2.78

TABLE 6

URINARY NITROGEN IN GRAMS

Rat No.	Diet									
	Control		50,000 rep		100,000 rep		500,000 rep		1,000,000 rep	
	Period I	Period II	Period I	Period II	Period I	Period II	Period I	Period II	Period I	Period II
1	1.04	1.39	1.12	1.68	1.56	1.47	1.38	1.37	0.89	0.98
2	1.12	1.61	0.99	1.40	0.90	1.21	1.06	1.40	0.82	1.03
3	1.42	1.32	1.46	1.77	1.38	1.83	1.32	1.59	1.13	1.49
4	1.42	1.67	1.40	1.59	1.32	1.63	1.47	1.60	0.86	1.28
5	1.44	1.61	0.84	1.16	1.39	1.41	1.78	1.28	1.07	1.37
6	1.33	1.36	1.20	1.09	1.05	1.21	1.25	1.41	1.35	1.81
7	1.10	1.28	1.18	1.37	0.99	1.39	1.11	1.30	1.40	1.61
8	1.40	1.58	1.21	1.46	1.16	1.47	1.31	1.45	1.24	1.01
Mean	1.28	1.48	1.17	1.44	1.22	1.45	1.33	1.42	1.09	1.32

TABLE 7

FECAL NITROGEN IN GRAMS

Rat No.	Diet									
	Control		50,000 rep		100,000 rep		500,000 rep		1,000,000 rep	
	Period I	Period II	Period I	Period II	Period I	Period II	Period I	Period II	Period I	Period II
1	0.53	0.43	0.51	0.43	0.54	0.58	0.50	0.51	0.33	0.47
2	0.41	0.43	0.60	0.76	0.54	0.53	0.45	0.64	0.40	0.33
3	0.59	0.57	0.52	0.61	0.48	0.67	0.46	0.49	0.55	0.47
4	0.68	0.69	0.62	0.53	0.56	0.58	0.51	0.53	0.54	0.38
5	0.57	0.55	0.49	0.60	0.59	0.60	0.66	0.51	0.54	0.60
6	0.52	0.50	0.38	0.42	0.50	0.50	0.46	0.46	0.56	0.61
7	0.57	0.59	0.55	0.58	0.42	0.47	0.45	0.46	0.73	0.64
8	0.70	0.69	0.71	0.71	0.48	0.56	0.39	0.45	0.53	0.57
Mean	0.57	0.56	0.55	0.58	0.51	0.60	0.49	0.51	0.52	0.51

TABLE 8

NITROGEN BALANCE IN GRAMS

Rat No.	Diet									
	Control		50,000 rep		100,000 rep		500,000 rep		1,000,000 rep	
	Period I	Period II	Period I	Period II	Period I	Period II	Period I	Period II	Period I	Period II
1	1.00	0.97	1.16	0.86	0.43	0.87	0.92	1.00	0.87	1.00
2	1.02	0.90	1.25	1.45	1.20	1.02	0.98	0.69	0.36	0.94
3	1.11	1.01	0.13	0.95	0.87	1.20	0.90	1.09	0.89	0.88
4	1.27	1.30	0.90	1.03	1.41	1.00	1.04	0.96	1.17	0.46
5	0.94	0.87	1.22	1.14	0.99	1.23	1.04	0.92	1.25	1.03
6	1.01	1.00	0.90	1.07	0.98	0.98	1.02	0.72	0.91	0.97
7	1.04	0.83	1.14	1.12	0.98	0.99	1.02	0.89	0.99	0.87
8	0.97	1.05	1.18	1.04	1.03	0.94	0.96	0.94	0.94	1.40
Mean	1.05	0.99	1.11	1.08	0.98	1.03	0.98	0.90	0.92	0.95

TABLE 9

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

Rat No.	Diet									
	Control		50,000 rep		100,000 rep		500,000 rep		1,000,000 rep	
	Period I	Period II	Period I	Period II	Period I	Period II	Period I	Period II	Period I	Period II
1	0.80	1.11	1.23	0.81	1.56	0.53	1.11	1.07	0.33	1.40
2	1.34	0.82	0.85	1.33	1.04	0.62	1.17	0.82	0.43	0.75
3	0.88	0.42	1.33	0.72	0.38	1.85	1.34	1.25	0.94	0.97
4	1.32	1.03	1.06	1.20	0.94	1.12	1.42	1.22	0.94	0.81
5	1.34	0.62	0.94	1.06	1.16	1.06	0.79	1.27	0.60	0.91
6	1.08	0.48	1.11	1.07	1.23	1.02	1.13	0.86	0.98	1.21
7	0.89	0.51	1.32	1.34	1.08	0.91	1.20	0.52	0.99	0.77
8	1.34	1.24	1.28	1.23	0.90	0.81	0.97	0.79	0.82	0.86
Mean	1.12	0.78	1.14	1.10	1.04	0.99	1.14	0.97	0.75	0.96

FIGURE 2

PROTEIN EFFICIENCY RATIOS, EXPRESSED AS WEIGHT GAIN IN GRAMS PER GRAM OF PROTEIN CONSUMED, FOR THE CONTROL DIET

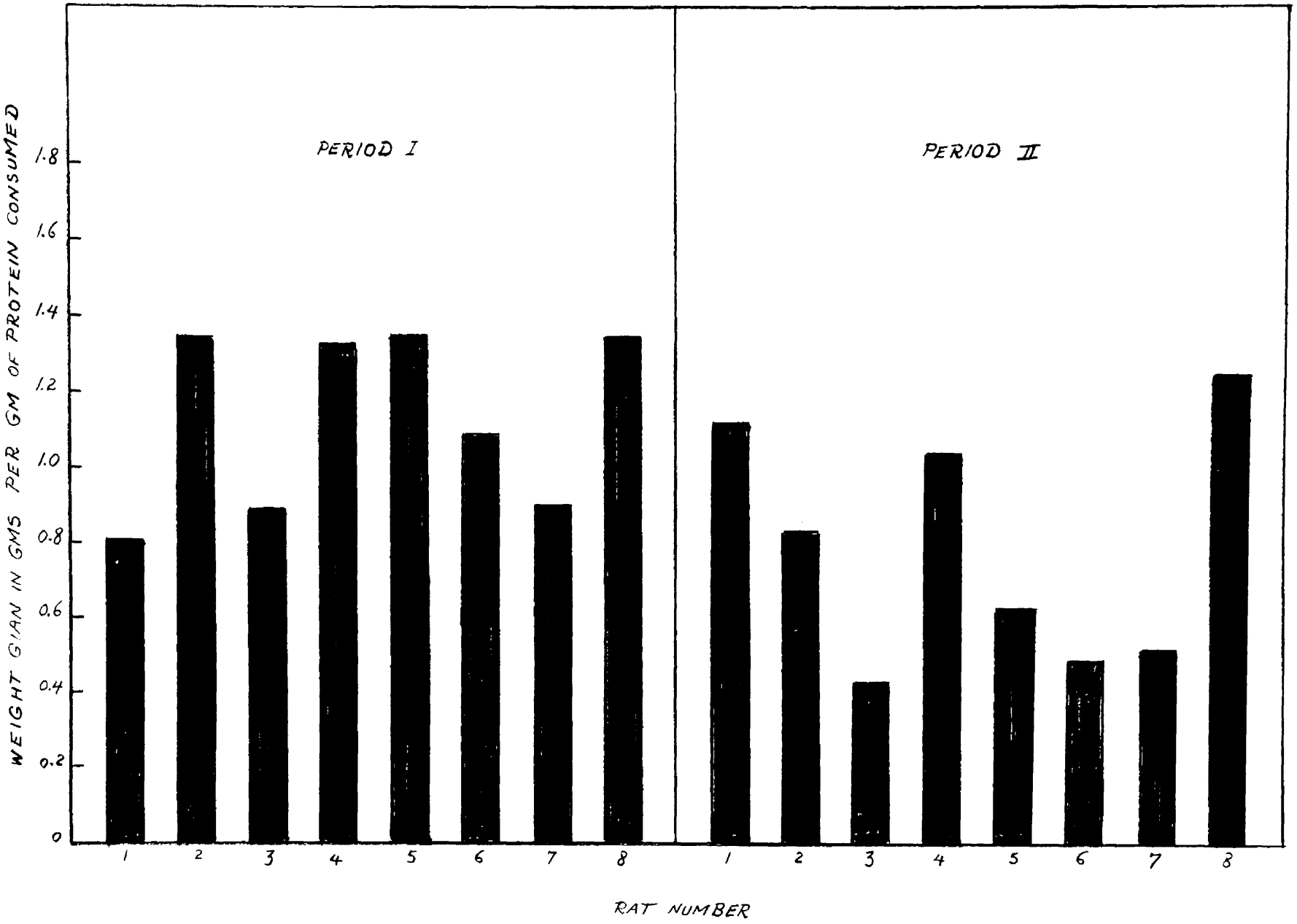
PERIOD I
PERIOD II
WEIGHT GIAN IN GMS PER GM OF PROTEIN CONSUMED
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
1
2
3
4
5
6
7
8
RAT NUMBER

FIGURE 3

PROTEIN EFFICIENCY RATIOS, EXPRESSED AS WEIGHT GAIN IN GRAMS PER GRAM OF PROTEIN CONSUMED, FOR THE 50,000 REP DIET

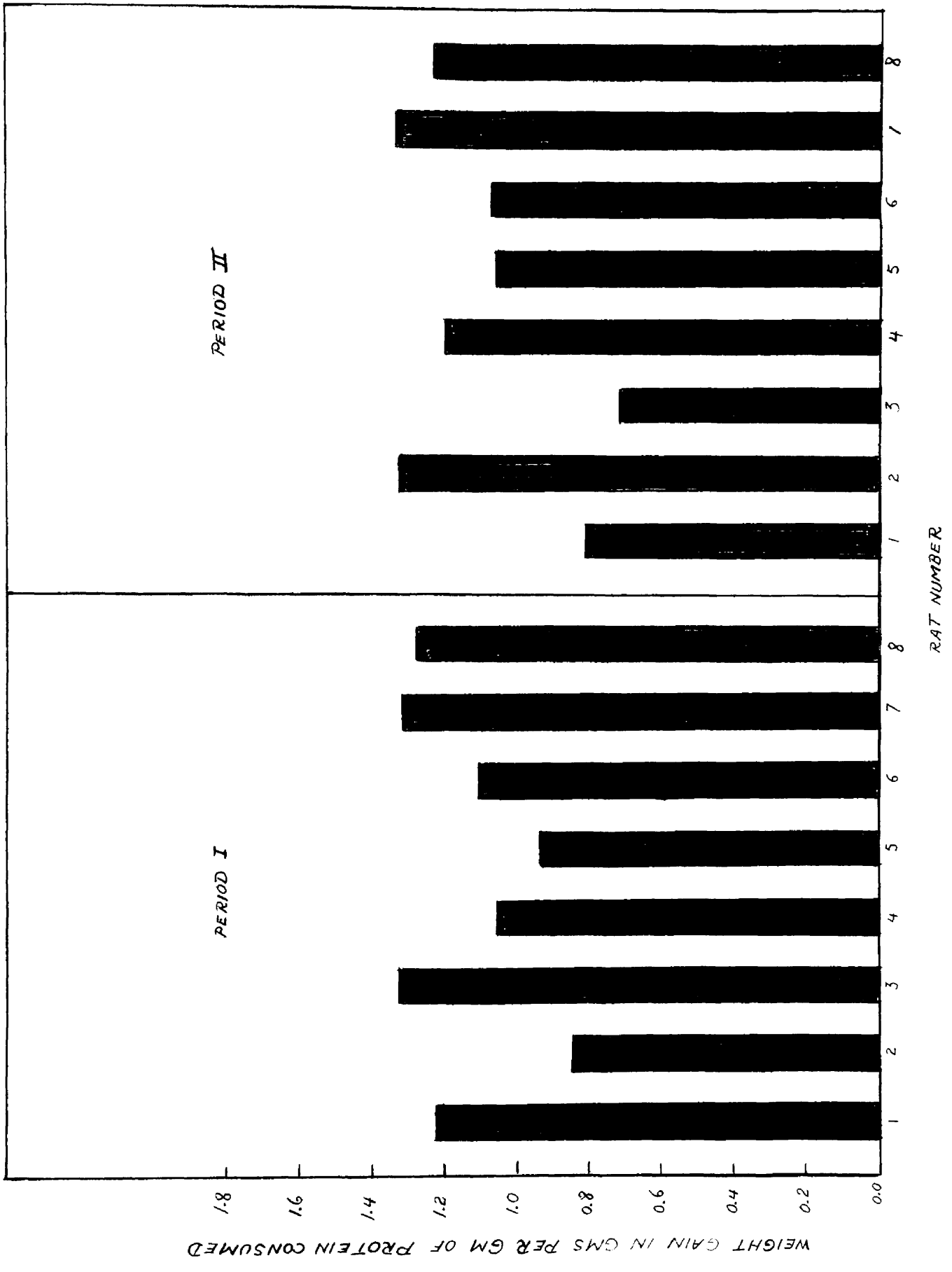
PERIOD I
PERIOD II
1
2
3
4
5
6
7
8
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
WEIGHT GAIN IN GMS PER GM OF PROTEIN CONSUMED
RAT NUMBER

FIGURE 4

PROTEIN EFFICIENCY RATIOS, EXPRESSED AS WEIGHT GAIN IN GRAMS PER GRAM OF PROTEIN CONSUMED, FOR THE 100,000 REP DIET

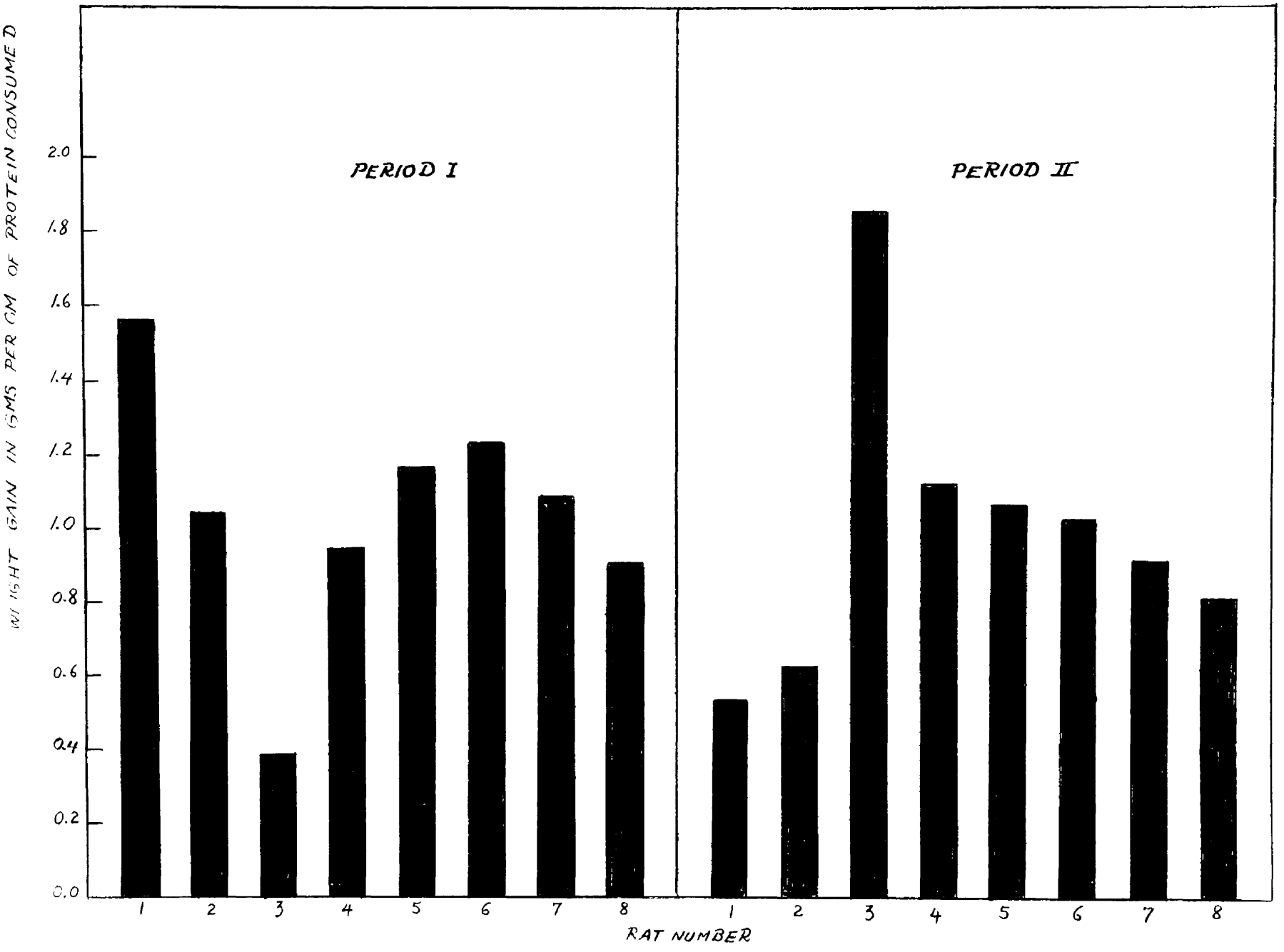
WEIGHT GAIN IN GMS PER GM OF PROTEIN CONSUMED
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
PERIOD I
PERIOD II
1 2 3 4 5 6 7 8
1 2 3 4 5 6 7 8
RAT NUMBER

FIGURE 5

PROTEIN EFFICIENCY RATIOS, EXPRESSED AS WEIGHT GAIN IN GRAMS PER GRAM OF PROTEIN CONSUMED, FOR THE 500,000 REP DIET

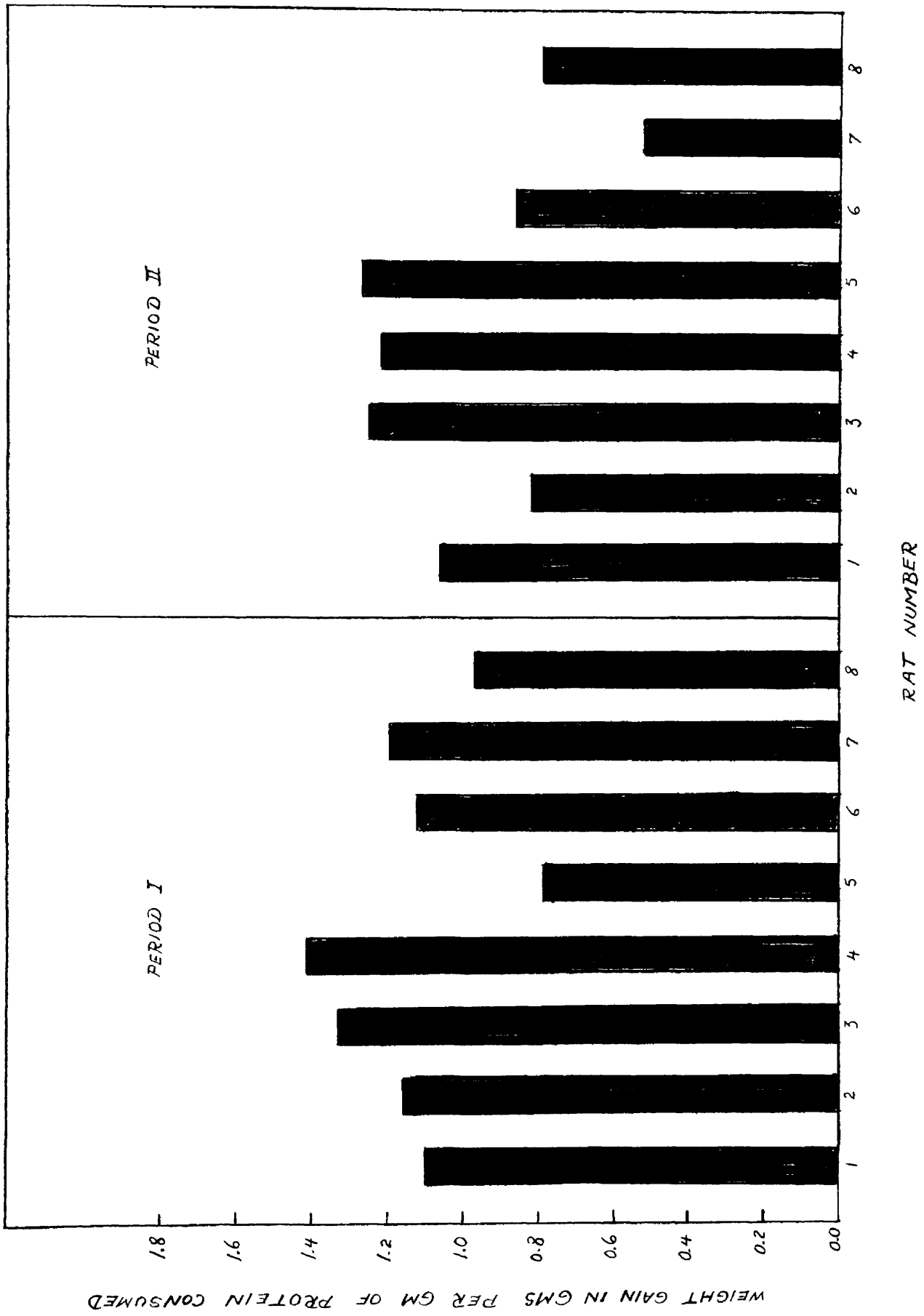
PERIOD I
PERIOD II
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
1
2
3
4
5
6
7
8
WEIGHT GAIN IN GMS PER GM OF PROTEIN CONSUMED
RAT NUMBER

FIGURE 6

PROTEIN EFFICIENCY RATIOS, EXPRESSED AS WEIGHT GAIN IN GRAMS PER GRAM OF PROTEIN CONSUMED, FOR THE 1,000,000 REP DIET

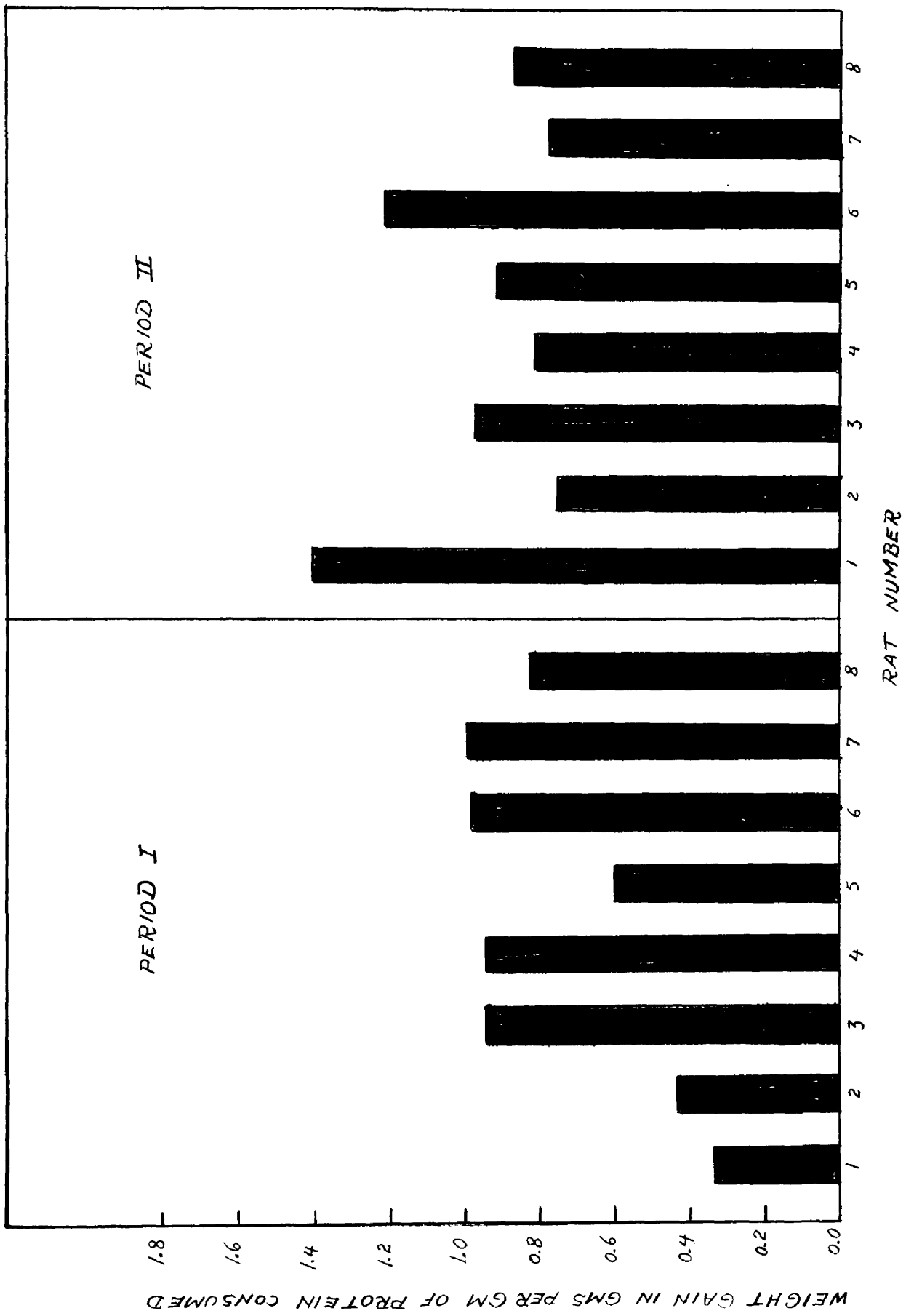
PERIOD I
PERIOD II
WEIGHT GAIN IN GMS PER GM OF PROTEIN CONSUMED
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
1
2
3
4
5
6
7
8
RAT NUMBER

TABLE 10

BIOLOGICAL VALUES
expressed as

$$\frac{\text{Nitrogen Intake - Nitrogen Output}}{\text{Nitrogen Intake}} \times 100$$

Rat No.	Diet									
	Control		50,000 rep		100,000 rep		500,000 rep		1,000,000 rep	
	Period I	Period II	Period I	Period II	Period I	Period II	Period I	Period II	Period I	Period II
1	39	35	42	29	17	30	33	35	42	41
2	40	31	44	40	45	37	39	25	23	41
3	36	35	36	29	32	33	34	34	35	31
4	38	36	31	33	43	31	34	31	45	22
5	32	29	48	39	33	38	30	34	44	34
6	35	35	36	42	39	36	37	28	32	29
7	38	31	40	36	41	35	39	34	32	28
8	32	32	38	33	39	32	36	33	34	47
Mean	36	33	39	35	36	34	35	32	36	34

TABLE 11

BIOLOGICAL VALUES
expressed as

$$\frac{\text{Nitrogen Intake - Nitrogen Output}}{\text{Nitrogen Intake - Fecal Nitrogen}} \times 100$$

Rat No.	Diet									
	Control		50,000 rep		100,000 rep		500,000 rep		1,000,000 rep	
	Period I	Period II	Period I	Period II	Period I	Period II	Period I	Period II	Period I	Period II
1	49	41	51	34	22	37	40	42	50	51
2	48	36	56	51	57	46	48	33	31	48
3	44	43	44	35	39	40	41	41	44	37
4	47	44	39	39	52	38	41	38	58	26
5	40	35	59	50	42	47	37	42	54	43
6	43	42	43	50	48	45	45	34	40	35
7	49	39	49	45	50	42	48	41	42	35
8	41	40	49	42	47	39	42	39	43	58
Mean	45	40	49	43	44	42	43	39	45	42

TABLE 12

CREATININE NITROGEN PERCENTAGES IN THE URINE

Rat No.	Diet									
	Control		50,000 rep		100,000 rep		500,000 rep		1,000,000 rep	
	Period I	Period II	Period I	Period II	Period I	Period II	Period I	Period II	Period I	Period II
1	4.62	4.37	4.74	4.29	3.65	4.29	3.95	4.53	5.28	4.86
2	4.39	4.38	4.20	4.11	3.86	4.21	4.69	4.54	5.44	4.66
3	3.81	4.23	4.26	4.22	3.76	3.62	3.94	3.76	5.16	4.30
4	4.95	4.53	4.36	4.30	3.51	3.60	3.68	4.07	3.79	4.05
5	4.39	4.57	3.55	3.51	3.93	3.99	3.28	4.21	4.09	3.85
6	4.28	4.11	4.20	4.42	4.07	4.05	4.06	4.80	4.28	4.27
7	4.42	4.05	3.82	3.67	4.52	4.07	4.35	4.19	3.56	3.90
8	3.76	3.58	3.96	4.34	3.60	3.91	4.07	3.91	3.94	4.04
Mean	4.33	4.23	4.14	4.11	3.86	3.97	4.00	4.25	4.44	4.24

TABLE 13

ANALYSIS OF VARIANCE[1] : F VALUES

	F Value	
	Among Diets	Among Collection Periods
Nitrogen intake	2.43	7.32**
Urinary nitrogen	1.83	18.63**
Fecal nitrogen	1.67	0.52
Nitrogen balance	2.16	0.22
Protein efficiency ratio	2.00	1.55
Biological value[2]	0.96	6.42*
Biological value[3]	1.31	8.13**
Creatinine nitrogen percentage in the urine	2.70*	0.03

*Indicates statistical significance at the 5% level.

**Indicates statistical significance at the 1% level.

[1]

Source of variance	d.f.
Total	79
Among diets	4
Among periods	1
Remainder	74

[2]Biological value expressed as:

$$\frac{\text{nitrogen intake - nitrogen output}}{\text{nitrogen intake}} \times 100$$

[3]Biological value expressed as:

$$\frac{\text{nitrogen intake - nitrogen output}}{\text{nitrogen intake - fecal nitrogen}} \times 100$$

TABLE 14

CORRELATION COEFFICIENTS

Comparison between Creatinine Nitrogen Percentage in the Urine and:	Correlation Coefficient
Protein efficiency ratio	- 0.17
Biological value[1]	+ 0.003
Biological value[2]	- 0.02

[1]Biological value expressed as:

$$\frac{\text{nitrogen intake - nitrogen output}}{\text{nitrogen intake}} \times 100$$

[2]Biological value expressed as:

$$\frac{\text{nitrogen intake - nitrogen output}}{\text{nitrogen intake - fecal nitrogen}} \times 100$$