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Protein nutrition and rates of turnover of serum globulins and antibodies

Julie Anderson Patten
Iowa State University

PROTEIN NUTRITION AND RATES OF TURNOVER OF SERUM GLOBULINS AND ANTIBODIES

by

Julie Anderson Patten

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Signature was redacted for privacy.
In Charge of Major Work

Signature was redacted for privacy.
Head of Major Department

Signature was redacted for privacy.
Dean of Graduate College

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INTRODUCTION

Malnutrition and chronic and acute infection are recognized to be serious and widely-prevalent health hazards today, particularly in tropical and technically underdeveloped areas of the world. Furthermore, the frequent association of famine and pestilence throughout history has led to the concept that nutritional deficiencies and infections are interrelated. The apparent relationship between infectious diseases and malnutrition, however, has proven to be quite complex and is not well understood.

A nutritional deficiency may result in a reduced frequency or severity of an infection, in which case it is referred to as an antagonistic reaction. For example, Boyd and Edwards (1963) observed an antagonistic reaction in chickens inoculated with Salmonella gallinarum or Newcastle disease virus as the level of dietary protein was altered. Increasing the level of dietary protein supplied by a mixture of corn and soybean meal supplemented with DL-methionine from 15% to 30% produced a simultaneous increase in mortality of the chickens. Conversely, a deficiency may cause an enhancement of the infection, or a synergistic reaction (Scrimshaw et al., 1959; Scrimshaw, 1966). Synergism between infections and nutritional deficiencies was demonstrated in mice by Schaedler and Dubos (1956). They found that mice exhibited a much increased susceptibility to tubercle bacilli, Staphylococci, or Friedländer bacilli when the

infective dose of each was administered at the end of a 48 hour fasting period. Even 24 hours of food deprivation had a profound infection-enhancing effect when animals had been fed an inadequate diet prior to fasting.

Malnutrition may be produced by either a relative or an absolute deficiency of one or more specific nutrients, or by a reduction in intake of all essential nutrients. Protein malnutrition alone or in conjunction with a deficiency in sources of energy is a common syndrome in nearly all technically underdeveloped countries in the world. Young children in these areas tend to be the most vulnerable to protein malnutrition and the high mortality rate in the 1- to 4-year age group is due largely to the synergistic interaction between protein malnutrition and infection (Béhar et al., 1958).

Protein and protein-calorie malnutrition are also evident among the adolescent and adult population of technically underdeveloped areas of the world (Viteri et al., 1964). East African adults suffering from protein deficiency had complaints of weakness, breathlessness and palpitations, and swelling of the ankles. In addition serum albumin, red blood cell count, serum pseudocholinesterase and protein-bound lipids were low compared to normal individuals. Although their normal diet was probably adequate in sources of energy, it consisted mainly of cassava, plantains and sweet potato which all contain 2% protein or less (Stanier and Holmes, 1954).

Studies of protein-deficient adults with respect to their resistance to infection have produced conflicting results. Wohl (1949) observed that patients with disturbances of protein metabolism sufficient to cause low concentrations of serum albumin, i.e., averaging 4.0 g/100 ml serum, showed an appreciable impairment in antibody response to typhoid immunization. Protein supplementation with lactalbumin hydrolysate or a modified casein preparation enhanced antibody formation in the hypoproteinemic patients; however, the high antibody titer of immune subjects with normal blood protein values was not attained. In contrast, Balch (1950) found no difference between nutritionally depleted patients and well-nourished adults in their ability to produce antitoxin to diptheria toxoid. However, only 10 of the 25 subjects had total serum protein below 6.0 g/100 ml serum and only 7 had serum albumin values below 2.7 g/100 ml serum. Likewise, Havens et al. (1954) observed that the anamnestic response of severely wounded men with low concentrations of serum albumin to diptheria toxoid was similar to that of healthy controls. However, the experimental depleted group of men averaged 6.8 g/100 ml and 3.3 g/100 ml serum for serum total protein levels and albumin levels which did not differ greatly from the average values of these parameters in the healthy controls, 7.4 g/100 ml and 4.3 g/100 ml serum, respectively. Scrimshaw et al. (1968), after reviewing studies on the interaction of protein deficiency and

infection, concluded that protein deficiency, if sufficiently severe, inhibits normal antibody response in children and adults and may also impair phagocytic activity, tissue integrity, inflammatory response, intestinal flora, endocrine metabolism, and non-specific protective mechanisms of the body.

Antibody production is one mechanism of resistance to infection that has been widely studied in an attempt to clarify the complex relationship between malnutrition and infection. The requirement for certain vitamins for antibody synthesis has been shown by Axelrod and Pruzansky (1955); however, the exact metabolic role of dietary protein remains uncertain.

Studies have shown that the qualitative characteristics of dietary protein or the relative proportions of dietary amino acids were as important as the quantity of protein consumed in conditioning resistance to infection (Dubos and Schaedler, 1958; Schaedler and Dubos, 1959). The world-wide prevalence of protein malnutrition emphasizes the importance of studies to clarify the basic relationship between protein nutrition and antibody production.

The nutritive quality of dietary proteins has been estimated by various methods involving a direct or indirect measurement of nitrogen retention in the body for maintenance or growth. In addition, indices developed from these data have been based upon body weight changes or changes in body nitrogen (Allison, 1964). Since the impact of protein nutrition is

especially marked in liver cells, changes in the composition of this tissue have frequently been used in evaluating dietary protein (Munro and Clark, 1960; Muramatsu and Ashida, 1962).

The concentrations of essential amino acids present in whole egg are often taken to be present in optimal proportions for use by the body; hence, the amino acid mixture in this protein source has been assigned a Biological Value of 100. Since egg proteins appear to be utilized most efficiently for protein synthesis during growth or tissue replacement, they should be helpful in investigating the relationship of dietary protein to the synthesis of antibodies and their rates of turnover.

The purposes of this study were 1) to evaluate diets containing various amounts of egg proteins on the basis of weight gain, ribonucleic and deoxyribonucleic acid in liver and splenic RNA and DNA, 2) to compare the effects of different levels of egg proteins on the immune response to sheep red blood cells, and 3) to determine the effect of different levels of egg proteins on rates of turnover of immunoglobulins. To compare the effect of various amounts of egg protein in the diet on antibody production, serum agglutinins and hemolysins were measured andrelated to the dietary intake. Specific activity of serum antibody and gamma globulin were determined so that turnover rates could be estimated. Concentrations of RNA and DNA in liver were obtained in order to assess hepatic capacity for

capacity for protein synthesis. In addition, splenic RNA and DNA were estimated for the purpose of evaluating the ability of this tissue to produce protein, in particular, gamma globulin and antibody.

REVIEW OF LITERATURE

The international importance of the relationship between nutritional status and infection has served as a stimulus for both experimental and clinical studies. The immune response or the production of antibodies is the measure of resistance to infection that has received the greatest attention in these studies. Although the need for certain vitamins and dietary amino acids for antibody synthesis has been established, the practical importance of the relationship still remains complex and obscure (Axelrod and Pruzansky, 1955; Schoenheimer et al., 1942).

The objective of this study was to relate weight gain and hepatic and splenic composition to production and turnover rates of immunoglobulins as a function of the quantity of dietary protein of high biological value. Thus, the following discussion will review the influences of dietary protein on serum antibodies, especially hemolysins and agglutinins, on turnover rates of serum globulins and antibodies, on splenic involvement in antibody production, and on several parameters indicative of the nutritional quality of dietary protein.

Production of Antibodies

Antibodies belong to the globulin fraction of blood serum. They are formed in cells of the lymphoid series, which appear to develop into plasma cells when subjected to suitable anti-

genic stimulation. The antibody-producing cells are found predominantly in lymph nodes, spleen, liver, and bone marrow.

Antibodies are produced in response to infection or to foreign substances introduced into the body. The unique property of an antibody is its ability to combine specifically with the substance or antigen that induced its formation.

Mechanism of antibody formation

Various hypotheses of antibody formation have been formulated; however, no one has received universal acceptance. Carpenter (1965) has grouped the different theories into two principal classifications: template hypotheses and selective hypotheses.

Template hypotheses: "The antigen serves as a pattern against which antibody globulin is synthesized or molded to produce a molecule possessing areas with a reverse structural image of determinant sites of the antigen (direct template), or else it modifies the DNA or ribonucleoprotein of globulin-producing cells so that they thereafter form globulin capable of reacting specifically with the antigen (indirect template)." Pp. 141-142.

Selective hypotheses: "Certain body cells are selected to produce antibody by virtue of an inherent or acquired specific reactivity with the antigen and are stimulated to do so by contact with the antigen. Several thousand cells with different specificity are destroyed in the embryonic state; those

that survive until immunologic maturity are the antibody-producing cells of the adult." P. 142.

The specificity of the immune response is satisfactorily accounted for by the template hypotheses while the selective hypotheses account for most of the observed features of the nature of antibodies and antibody production.

Sites for antibody formation

Studies have shown that antibodies can be formed in the spleen, lymph nodes, bone marrow, lungs, liver and skin following injection of antigen. However, the spleen and lymph nodes appear to be the major sites of antibody production (van Furth et al., 1966; Harris and Harris, 1950; Keston and Katchen, 1956). The lymph node cells appear to bear the principal burden of antibody production following local injection of antigen; whereas spleen cells are important sources of antibody following intravenous injection of antigen (Harris et al., 1954; Wissler et al., 1953; Rowley, 1950).

Studies concerning the identity of cells that produce antibody have indicated the involvement of a variety of cells such as macrophages, lymphocytes, plasma cells and large pyroninophilic cells. Part of the confusion or disagreement among investigators as to the identity of antibody-producing cells may be due to the terminology used in identifying the cells and to the interrelationship existing between these cell types. Transitional stages have been observed between small and large

lymphocytes and plasma cells (McMaster, 1953). Reticular cells also may be converted into large, pyroninophilic, antibody-producing cells, some of which eventually mature as adult plasma cells; however, the majority develop into lymphocytes (Wissler et al., 1957). Fluorescent antibody studies of lymph nodes demonstrated the presence of antibody first in immature cells which later differentiated into mature plasma cells possessing higher antibody concentrations (Leduc et al., 1955). Nossal et al. (1963) concluded from studies of the primary immune response in lymph nodes that in the primary response primitive lymphoid cells are induced into plasma-cytopoiesis by antigen.

Immature plasma cells in the red pulp of rabbit spleen have been indicated as the chief source of antibody during the primary and secondary responses to intravenous injection of antigen (Fagraeus, 1948 and Langevoort et al., 1963). However, Gunderson et al. (1962) observed proliferation of large pyroninophilic cells in the red pulp of rat spleen along with a brisk antibody response to intravenous injections of 5% sheep erythrocyte antigens. Also non-phagocytic, mononuclear cells and plasma cells in the red and white pulp of the spleen, respectively, have been shown to produce antibody during the primary response to intravenous antigen injections (Schoenberg et al., 1965).

Cannon and Wissler (1967), utilizing autoradiographic techniques, obtained further evidence of the active proliferation of large pyroninophilic, "antibody-forming" cells in the splenic red pulp following a single intravenous injection of particulate antigen. In addition their results strongly suggested that these cells appear in large numbers in the red pulp at the peak of the immune-induced splenic hyperplasia. Both the large pyroninophilic cells and the descendent lymphocytes were observed to migrate from the spleen into the blood to be distributed to lymph nodes and bone marrow. They concluded that this migration of antigen-stimulated spleen cells may be important in the dissemination of immunological information.

The thymus has been associated with the immune process; however, its exact function has not been elucidated (Harris et al., 1948; Miller, 1962). Van Furth et al. (1966) found antibody-forming cells in thymus tissue, but concluded that these cells originated elsewhere and were only trapped in the thymus from the circulation. Recent studies have indicated a synergistic relationship between thymus, bone marrow, and spleen cells with respect to their functions in antibody production in immune mice. Irradiated mice injected with a suspension of syngenic normal thymus, spleen, and bone marrow cells produced more centers of hemolytic activity in their spleens following immunization with sheep erythrocytes than mice receiving either a combination of spleen and thymus cells only or one of spleen and marrow cells. Thymus cells from normal and from immune

animals or normal marrow cells alone produced little or no hemolytic activity in irradiated immunized mice (Claman et al., 1966a and Claman et al., 1966b).

Radovich et al. (1968) observed that the injection of 5 million bone marrow cells into cobalt-60 irradiated mice produced an increase in spleen size and greatly enhanced the number of antibody-forming cells found in the spleen 6 days after injection of sheep erythrocytes and small numbers of spleen cells from normal or preimmunized mice. These investigators interpreted their data to indicate a nonspecific effect of bone marrow cells on localization or proliferation of antibody-forming cells in the spleen. In addition, their findings were considered to negate previous claims that bone marrow enhancement could be considered evidence for a multipotential antibody-forming cell precursor.

Another view has been presented by Mitchell and Miller (1968) who concluded from studies of the immune response in mice that the immediate precursor of the 19S hemolysin-forming cells was marrow-derived. They suggested that thymus or thoracic duct lymphocytes recognized an antigen and interacted with it in some way that triggered differentiation of the essentially passive bone marrow-derived precursor cell to a specific antibody-forming cell.

Chaperon et al. (1968) in their studies on the relationships among the spleen, thymus, and bone marrow during the

immune response to sheep red blood cells observed that production of 19S antibody in mice reached a peak 4 days after injection of sheep erythrocytes and declined rapidly thereafter. Cells producing 19S antibody were found in spleen but not in thymus or bone marrow tissue and apparently did not circulate, at least not within their lifespan as producers of 19S antibody. Seven days after immunization, peak 7S antibody production occurred; cells responsible for this were more numerous in the spleen than in other locations and persisted in the body for several weeks. These cells were believed to circulate since increasing numbers of them were found in the thymus and marrow with increasing intervals after immunization. In addition, these investigators studied the presence of antigen-sensitive precursor cells (memory cells) in thymus, bone marrow, and spleen tissue by transferring aliquots of cells from these tissues obtained from immune mice to irradiated recipients. After stimulating the recipients with the antigen, plaque-forming cells in their spleens were counted. The pattern of appearance and distribution of the memory cells was similar to that of the 7S producers in immune mice, i.e., they appeared first in spleen tissue and later in thymus and marrow tissue. Hence, Chaperon et al. suggested that thymus and marrow cells from immune mice contained a substantial proportion of immigrant cells, apparently derived from the spleen.

Characteristics of antibodies

The properties of antibodies differ, but some general statements can be made which apply to all types. Antibodies have been identified as proteins possessing the solubility of globulins. They migrate electrophoretically with the gamma globulins or as distinguishable components of the beta and gamma globulins. The molecular weights of antibodies fall into two principal classes: approximately 160,000 or "normal globulin" and 1,000,000 or "macro-globulin". The sedimentation constants associated with these two classes are approximately 7S and 19S, respectively.

General agreement exists that in human, and probably other mammalian serum, three main antigenically distinct types of globulin carry antibody activity. In the absence of universal terminology for them, the following alternative names for the three major types were summarized by Kabat and Mayer (1967):

i. IgG, γ_{2-}, 7S, or γ-globulin

ii. IgM, γ_1M-, 19S, or β_{2M}-globulin

iii. IgA, γ_1A-, or β_{2A}-globulin

Banovitz and Ishizaka (1967), using radioimmunoelectrophoresis, detected five components having antibody activity in rat antisera: γ_1-, γ_2-, and γ_M-globulins and two unidentified components, U_1 and U_2. The investigators identified γ_1-globulin as γ_A-globulin reported by other workers or as a second γ-globulin or γ_2-globulin.

The only known consistent and detectable distinction between normal γ-globulin and antibody is the specific immunologic reactivity of antibody to antigen (Carpenter, 1965). During antigen-antibody reactions, the two kinds of molecules join at their complementary determinant sites in a lock-and-key manner, gradually forming a three-dimensional network or lattice in which antigen and antibody alternate.

The nature of the antigen and the antibody, the presence of other cells or substances, the physical conditions, and other factors determine the specific result observed. Particulate antigens such as bacteria and erythrocytes agglutinate or clump together when mixed with homologous antibodies, termed agglutinins, under proper conditions (Carpenter, 1965). Lysis of the cells may occur if complement, a normal serum component, is present during the reaction. Hemolysis is the specific term applied to the dissolution of erythrocytes and hemolysin is the specific antibody involved.

The immune reaction may be a protective process when it assists in overcoming infection or a destructive process when it produces hypersensitivity or immunologic disease.

Synthesis and metabolism of antibodies

Antibodies are synthesized de novo from free amino acids rather than from more complex precursors (Keston and Katchen, 1956; Stavitsky, 1958). Antibody synthesis has been shown to follow the same pattern as mammalian protein synthesis in

general (Strander, 1966; Haurowitz, 1965; Smiley et al., 1964).

The immune reaction generally can be differentiated into two different responses, the primary or initial response and the secondary or anamnestic response. Primary antigenic stimulation is usually followed, after an induction period of several days, by slight to moderate liberation of antibody into the circulating blood. If no further contact with the antigen occurs, the antibody titer then decreases, rapidly after immunization with erythrocyte antigens and slowly with bacterial antigens. A subsequent or second injection of the same antigen after suitable time interval produces a more rapid rise in antibody titer, often to a higher level which is maintained longer than that of a primary response (Dixon et al., 1954; Nossal and Makela, 1962). However, the secondary response of rats to sheep erythrocyte antigens was observed to be similar to that of the primary response, i.e., a rapid rise followed by a rapid fall in titer, except that the low titer was maintained for a longer period of time in the secondary response than in the primary (La Via, 1964). The antibody response to antigenic stimuli varies with the nature of the antigen; the dosages, number, frequency and route of injections; and the species as well as individual animals within a species (Carpenter, 1965).

Campbell and La Via (1967) noted that the normal response of rats to a single intravenous injection of sheep erythrocytes was a rise in antibody titer until day 6 followed by a decrease in titer to a baseline level.

The primary immune response generally has been characterized by the initial appearance of 19S immunoglobulin in the circulation followed later by 7S antibody which persists for a period of time. Both 19S and 7S antibodies have been observed in the secondary response; however, 7S antibody usually appeared to be the dominant species with the passage of time (Bauer and Stavitsky, 1961; Uhr and Finkelstein, 1963; Bauer et al., 1963; Nossal et al., 1964; Adler, 1965).

Rabbit 19S antibody formed early to sheep erythrocytes possessed most of the hemolytic activity while the combining or agglutinating activity was found chiefly in the 7S antibody produced later (Talmage and Taliaferro, 1954; Stelos and Talmage, 1956 and 1957). Adler (1965) observed that during the immune response of mice to intraperitoneal injections of sheep erythrocytes the ratio of hemolysins to agglutinin titers changed from about 10:1 on day 4 to 1:3 late in the primary response and eventually to 1:8 late in the secondary response. Since 7S antibody was more active in agglutination than in hemolysis, the change in the ratio of titers reflected a replacement of 19S antibody by 7S antibody. The relative hemolytic efficiency of 19S antibody was not constant because it decreased with time during the primary response, increased temporarily during the initial secondary response and then declined again.

Since antibodies have been associated nearly exclusively with the gamma globulin fraction of the plasma, antibody production has been investigated generally as an aspect of plasma

protein synthesis. The biosynthesis and metabolism of gamma globulin in particular have been studied to gain information applicable to antibody metabolism.

For a number of years, especially since the advent of the isotope tracers, body proteins have been characterized as dynamic, i.e., continually synthesized and degraded. To quantify this dynamic state, the turnover rate has been defined as the quantity of protein destroyed or newly synthesized per unit of time (Schoenheimer et al., 1942). Protein turnover has also been considered as the replacement of an amount of protein by an equal quantity of protein either newly synthesized from its metabolic precursors or transported into the system from outside (Neuberger and Richards, 1964).

Since plasma concentrations of antibody reflect rates of synthesis and catabolism of antibody, complete characterization of the immune response necessitates a measurement of the turnover rates of antibodies. Investigation of the overall rate of protein synthesis in man has demonstrated that individual proteins turned over at widely different rates with the plasma proteins being among the most active fractions (San Pietro and Rittenberg, 1953). Turnover of proteins resulted from degradation of intact protein molecules rather than exchange of amino acids in otherwise intact protein molecules (Penn et al., 1957; Walter and Haurowitz, 1958).

When studying turnover of serum proteins and hence of antibodies, one must consider their equilibration between the liquid, physically homogeneous serum and the less homogeneous, extravascular space. The extravascular space is composed of many compartments such as the fluids of serous cavities and joints, cerebrospinal, labyrinthine and eye fluids, intestinal lymph, interstitial tissue fluid, and serous material that serves as a solvent for most bodily secretions. In man the protein content in extravascular spaces, and in the lymph which drains them, may range from as little as 20 mg/100 ml in the cerebrospinal fluid to as much as 5 g/100 ml, i.e., 70% of the plasma protein concentration, in hepatic, intestinal and thoracic-duct lymph (Schultze and Heremans, 1966). The mass of extravascular protein in equilibrium with the plasma is at least as great and often even greater than that present in the blood vessels. A normal 70-kg adult has a circulating mass of intravascular albumin equal to 110-140 g, whereas an additional quantity of about 190 g of albumin is present in the interstitial tissue spaces (Schultze and Heremans, 1966). Hence, slightly more than half of the total exchangeable albumin pool, estimated to range between 250 and 300 g, is located in the extravascular pool.

Immunoglobulins differ from albumin somewhat in their distribution in the body. In man the high-molecular weight constituent γ_1M-immunoglobulin (IgM) appears to be concentrated mainly in the intravascular pool, about 82% of the body content,

while γG-immunoglobulin (IgG) appears about equally distributed over the plasma and extravascular spaces, 40% intravascular and 60% extravascular (Barth et al., 1964). The third main component of the antibody system, γA-immunoglobulin (IgA), appears to be distributed similarly to γG-immunoglobulin (Schultze and Heremans, 1966). Costea et al. (1962) estimated the total exchangeable pool of γM-immunoglobulin in man to be 7.8 g, the intravascular pool to be 5.1 g, and the extravascular pool to be 2.7 g. Likewise, the total exchangeable, intravascular, and extravascular pools of γG-immunoglobulin were estimated to be 42.2 g, 19.8 g, and 25.4 g, respectively.

Cohen (1957), utilizing iodinated serum proteins in 250 g rats, estimated the mass of circulating albumin to be approximately 385 mg and gamma globulin to be 83 mg. The extravascular/intravascular ratio for albumin was approximately 1.01 and for gamma globulin, 1.89; therefore, the total exchangeable pool for albumin was about 774 mg and gamma globulin about 240 mg. Jeffay and Winzler (1958a) obtained similar estimates with the use of ^{35}S-labeled plasma proteins in 200 g rats. They calculated that the average size of the exchangeable pool of albumin was 3.1 mg/g of body weight or 620 mg/rat and of gamma globulin, 1.5 gm/g body weight or 300 mg/rat.

Different rates of equilibration between the intravascular and extravascular pools of antibodies have been observed in rabbits immunized with sheep erythrocytes (Taliaferro and Talmage, 1956). Antibodies with small molecular weights

(157,000 to 165,000), with both Forssman and isophile specificity, and with a slow rate of metabolic decay, equilibrated initially in 1.1 to 1.8 days during which time up to 60% of this class of antibodies left the blood. However, antibodies with large molecular weights (900,000), with both Forssman and isophile specificity, and with a high rate of metabolic decay equilibrated in shorter periods, 0.3 to 0.6 days, during which time less antibody disappeared from the blood, 18% for the Forssman type and 45% for the isophile type.

Turnover rates from disappearance of serum radioactivity data

Turnover rates of plasma proteins have been determined experimentally either by incorporation of labeled amino acids into proteins or by the disappearance of labeled plasma proteins from the circulation. The observed apparent rates have changed with the duration of the experiments and usually have been considered to be the sum of several first order processes. The exchange of plasma proteins between the intravascular and extravascular spaces may account for at least one of the first order processes while reutilization of labeled amino acids which were liberated from other body proteins may be responsible for another (Campbell et al., 1956; Penn et al., 1957; San Pietro and Rittenberg, 1953; Zilversmit and Shore, 1952). After correction for various first order rate processes, the experimental data appeared to justify the conclusion that turnover of plasma proteins, in general, was also a first order process (Anker, 1960).

Turnover rates of plasma proteins generally have been measured by determining the rate of disappearance of radioactively labeled plasma proteins from the circulation. Disappearance of radioactivity from plasma proteins after a single administration of a labeled amino acid, however, cannot be equated with the true rate of turnover of the plasma protein fraction on which the measurement was carried out (Anker, 1960 and McFarlane, 1957). When labeled amino acids were administered, approximately 5% of the dose was incorporated into plasma proteins, part of the remainder was excreted and part incorporated into tissue proteins. Hence, a substantial store of labeled amino acids was present in the body tissues which were gradually released by catabolism and became available for incorporation into plasma proteins. This process, termed reutilization, must result in an increase in the apparent half-life of any protein since a labeled molecule may be removed and replaced by another labeled molecule instead of by an unlabeled one.

The turnover rate of plasma proteins also depends on the particular labeled amino acid used since different amino acids when fed simultaneously to the same animal have been eliminated with different apparent turnover rates from a single protein (Penn et al., 1957). For example, rate constants or rates of isotope elimination for some individual amino acids during the exponential period of the decay curve of serum albumin were lywine 0.044, leucine plus valine 0.050, arginine 0.056, alanine 0.064, and glycine 0.066 units/day relative to an initial iso-

tope concentration of one. The measured rates were the differences between the rate of breakdown of serum albumin and the rates of reutilization of the individual amino acids.

Isotope concentrations of amino acids released from proteins of the body depend on dilution by dietary and biosynthetic unlabeled amino acids; thus, the quantities of isotope amino acids reincorporated into plasma proteins some time after initial administration will vary with different amino acids. The essential amino acids were observed to have lower apparent turnover rates, i.e., their labeled forms derived from the body proteins were less diluted than those of non-essential amino acids (Penn et al., 1957). These investigators suggested that the essential amino acids were diluted from dietary sources alone while non-essential ones were diluted by both dietary and biosynthetic supplies.

The measurement of the rate of disappearance of the isotopic tracer from a labeled plasma protein fraction after transfusion into a recipient organism has yielded more consistent experimental data, although variation still occurs among investigators. Dixon et al., (1952 and 1953), using iodinated plasma fractions in dogs, obtained half-lives for albumin and gamma globulin of 8.2 and 8.0 days, respectively. Loss of serum radioactivity was followed for 17 days in gamma globulin measurements and for 28 days in albumin measurements. Goldsworthy and Volwiler (1958), using plasma proteins doubly

labeled with cystine-^{35}S, lysine-^{14}C, obtained average apparent half-lives for albumin and gamma globulin in the dog of 16 days and 19 days, respectively. These workers had followed the loss of the isotopes from the serum for 60 days and graphically resolved the plasma radioactivity decay curve into several rate processes. They used the slowest rate process or component of the decay curve to calculate these apparent biological half-lives of the isotopes in the protein molecule. The long apparent half-lives of albumin and gamma globulin obtained by Goldsworthy and Volwiler may have been due to isotope reutilization which probably became significant during the second half of the experiments from which their data were obtained.

Data obtained using ^{35}S-labeled plasma proteins transfused into rats indicated that the half-life of albumin was 3.7 days and that of gamma globulin, 5 days (Jeffay and Winzler, 1958a). Campbell et al. (1956), using ^{131}I-labeled plasma proteins, obtained half-lives in the rat of 3.1 to 3.5 days for albumin and 6.0 to 7.4 days for gamma globulin. Calculations of the biological half-lives in both these studies accounted for equilibration between the extravascular and intravascular pools.

The variability in the turnover rates obtained for the plasma proteins by the different investigators may be due predominantly to the wide variation in experimental procedures followed. In addition, the nutritional status of the experimental animal may affect significantly the turnover rates of

the plasma proteins. Picou and Waterlow (1962), studying albumin metabolism with ^{131}I-albumin in malnourished Jamaican infants, found that the fractional catabolic rate of albumin, calculated from ^{131}I urinary excretion, was significantly less in the malnourished state than after recovery. Hence, the half-life of albumin was longer during the period of malnutrition than after recovery.

Urinary radioactivity resulting from the metabolic breakdown of labeled molecules in the plasma is easily measured and can be used to estimate plasma protein turnover (Schultze and Heremans, 1966). The assumption that the destruction and renewal of the plasma proteins, takes place exclusively in or very near to the plasma compartment is based on the fact that, for albumin at least, the urinary excretion rate of the label is at all times proportional to the concentration of label prevailing in the plasma over the same period of time (Berson and Yalow, 1954). Further support of this assumption is the fact that the urinary excretion rate bears no relationship to the corresponding concentrations in the whole body, nor to those computed for the extravascular lymph space (Schultze and Heremans, 1966). On the other hand, present knowledge suggests that some part of the catabolism of gamma globulin may take place outside the vascular system since close parallelism between rate of urinary excretion and decay of activity in the plasma is not found with gamma globulins (Schultze and Heremans,

1966). The influence of an organism's nutritional state with respect to protein on turnover rate of serum proteins will be discussed later.

Humphrey and McFarlane (1954) observed that the specific radioactivities of transfused labeled antibody and globulin in the plasma of immune rabbits both declined at about the same rate, 12.5% per day, i.e., half-lives of about four days. Andersen and Bjørneboe (1964) studying the turnover of ^{131}I-gamma globulin in rabbits before and during hyperimmunization with pneumococci vaccine, calculated the half-life of gamma globulin to be 5.5 days before immunization and 3.5 days after immunization.

Turnover rates from radioactivity incorporation data

The rate of incorporation of labeled amino acid into plasma protein fractions has been utilized to provide an estimate of their rates of synthesis and, hence, an indication of their turnover rates. Green and Anker (1955) noted in rabbits that serum proteins were labeled about twenty minutes after injection of labeled amino acids and maximum labeling occurred in 50 to 75 minutes. A similar time course for initial labeling of rabbit plasma proteins, including antibody globulin, was also observed by Gregoire et al. (1958); but the labeled plasma fractions reached maximum concentration 4 to 5 hours after administration of the labeled amino acids. Labeled serum albumin and globulin in rats appeared about 18 minutes after

administration of tracer and rose sharply to a plateau in 45 to 50 minutes (Peters, Jr., 1962). Humphrey and Sulitzeanu (1958) found that, following a single intravenous injection of labeled amino acids into hyperimmune rabbits, intracellular antibody became labeled almost immediately in the lungs, bone marrow, spleen, lymph glands, and appendix. Maximum labeling of intracellular antibody had occurred after 15 minutes and remained at that level for 1 to 2 hours, after which it slowly declined. However, in the plasma of the same animals labeled antibody was not detected until 20 to 25 minutes after administration of the label and maximal values occurred between 2 and 4 hours afterward.

Other investigators have observed similar time sequences for incorporation of isotopic tracers into intracellular and extracellular antibody. The secretory lag or the time interval before labeled antibody appears in the extracellular medium is believed to reflect the translocation of newly synthesized antibody from the cell sap into the surrounding medium (Helmreich et al., 1961; Ogata et al., 1961).

Differences in the rate of incorporation of radioisotopes into plasma proteins may be due in part to the particular labeled amino acid used. Variation in the characteristics of cellular uptake among different amino acids have been observed and related to physical and chemical properties of the particular amino acid (Christensen, 1955). Manchester and Wool

(1963) observed that glycine exhibited a slow initial penetration and a long-continued accumulation into rat heart muscle while aromatic amino acids such as phenylalanine and tyrosine were taken up rapidly and quickly reached a constant concentration. Entry of labeled amino acids into intracellular water was observed within minutes and continued to rise for as long as 80 minutes for some amino acids but reached a constant concentration in 10 minutes for others. Lysine was observed to have the lowest ratio of intracellular/extracellular concentration of all the amino acids. In addition, the concentration in plasma proteins of a particular amino acid might also affect the time required for maximum incorporation of the labeled acid into protein (Penn et al., 1957).

Examination of immunoelectrophoretic patterns of plasma proteins labeled in vitro by mouse tissues has shown that radioactivity in immune globulins increased when tissues were obtained from mice infected with staphylococci (Williams et al., 1963). Williams (1965) also found that mice, injected intravenously with ^{14}C-labeled amino acids four days after intravenous infection with staphylococci, incorporated 5% of the label into the circulating serum proteins in 2 hours, while 1% incorporation occurred in normal animals. The incorporation rate of the label into a crude albumin fraction of infected mice was half that of controls while incorporation into crude globulins and the immune globulin fraction was ten times more

rapid. From maximum levels the decline in labeled protein was significantly more rapid in infected animals than in controls, being reduced by half in 24 hours. The concentration of serum proteins, however, increased approximately 50% during 5 days of infection.

Gill and Gershoff (1967) noted that not all of the gamma globulin formed in Cebus monkeys following immunization with a synthetic polypeptide antigen was antibody. The percentage of antibody in the gamma globulin fraction also varied under different circumstances. The primary and early responses produced an increase in total gamma globulin as well as an increase in antibody. In the late secondary response concentration of gamma globulin remained constant, but the amount of antibody in it increased.

Investigation of protein metabolism suggested that plasma protein turnover was mainly a first-order reaction based on constant fractional rates of conversion of amino acids to proteins in conjunction with equally constant fractional protein catabolic rates. Neuberger and Richards (1964) have listed the following assumptions which were necessary for the interpretation of turnover data obtained from isotopic incorporation studies:

1) The administered labeled amino acid must be given in a sufficiently small quantity, so that normal metabolism of the compound is unaltered.

2) The labeled amino acid used as a tracer must penetrate freely to the locus of protein formation.
3) Cells should not contain any quantitatively important stores of amino acid derivatives which are intermediates in protein synthesis and which might dilute the label.
4) Proteins are formed from amino acids; when proteins are catabolized the amino acids released mix completely with the free amino acids in the cell, i.e., there is no preferential reutilization of amino acids or peptides derived from protein breakdown.
5) The living organism will metabolize labeled amino acids in exactly the same way as unlabeled amino acids.

Dietary Protein and Antibody Production

Dietary protein and resistance to infection

Resistance to infection has been related specifically to protein nutrition by numerous investigators. Dubos and Schaedler (1958) found that weanling mice fed an 8% casein diet were more susceptible to bacterial infections than mice receiving a diet of 20% casein supplemented with cystine. Mice fed pellets containing a minimum of 21% crude protein from many sources, mainly of vegetable origin, were intermediate in resistance to the bacterial infections. However, after the 8% casein diet was supplemented with a 12% amino acid mixture

which had been shown to allow rapid regeneration of blood constituents in mice subjected to repeated bleeding, mice fed this diet became the most resistant to infection. Mice fed both supplemented casein diets also gained weight somewhat more rapidly than did those fed pellets. Therefore, the proteins provided by pellets, derived mainly from vegetable sources, were probably inferior to the supplemented casein in nutritional value. The fact that animals fed pellets were more susceptible to infection than those fed 20% casein supplemented with cystine or 8% casein plus the amino acid mixture implied that the qualitative characteristics of the dietary protein were as important as the quantity of protein fed.

When either soybean alpha-protein or wheat gluten was substituted for casein, in concentrations of 15% or 20% dietary protein, mice were susceptible to bacterial infection (Schaedler and Dubos, 1959). However, mice fed a mixture of soybean and rice flour providing 15% protein and an amino acid pattern similar to that of casein resisted infection to the same extent as animals on a 15% casein diet and were more resistant than animals fed 15% gluten. Also the infection-enhancing effect of low levels of dietary casein, 5 and 8%, could be corrected by supplementation with an amino acid mixture designed to approximate the composition of the 15% casein diet. Thus the relative proportions of various dietary amino acids appeared to be as important a factor as their total amount in conditioning resistance to bacterial infection.

Hill and Garren (1961) found that raising dietary protein, supplied as either soybean meal or casein, from 10% to 20% or 30% resulted in a progressively increased rate of mortality of chicks from Salmonella gallinarum infection. At the end of the experiment differences in total mortality between 20 and 30% protein levels from casein were statistically significant; however, differences between 10, 20, and 30% soybean meal were not.

Boyd and Edwards (1963) observed that chicks inoculated with Escherichia coli suffered greater mortality on 15% than on 30% dietary protein supplied by a mixture of corn and soybean meal supplemented with DL-methionine, while those inoculated with Salmonella gallinarum or Newcastle disease virus suffered greater mortality on the 30% dietary protein diet. Therefore, the infecting agent was a determining factor in the response of an organism to infection.

Growing chicks fed a 22% protein diet containing sesame meal supplemented with lysine showed a higher mortality rate from injected Newcastle disease virus than chicks fed a diet containing 28% protein from the same source. Thus, a beneficial effect was realized from protein intakes beyond that necessary for optimal body weight gains which were obtained on the 22% protein diet (Fisher et al., 1964).

These studies and others have demonstrated variability in the response of experimental animals to the interaction of protein nutriture and infection. A number of circumstances

could explain this variability. First, protein deficiency may not have been sufficiently severe or prolonged to influence the course of the infection. Secondly, wide variations in nutritional status may have failed to exert a detectable influence on an infection if the hosts have such high natural resistances to the infection that none is seriously affected or, conversely such a low natural resistance that all succumb. Thirdly, when viral infections are involved, protein deficiency may affect the infecting agent more than the host, with a resulting antagonism rather than synergism.

Nutritional and genetic factors affect the natural resistance of mice to Salmonella infections (Schneider, 1956). A Salmonella resistance factor, which enhanced the natural resistance of mice to Salmonella infections, has been isolated from wheat, malted barley, and commercial dried egg white. This nutritional resistance factor appeared to be in dynamic equilibrium in the host and its action was decisive in the early hours of the infection. On the basis of this work, Schneider suggested that, in the natural world, nutritional entities exist which are capable of enhancing natural resistance to infection and which are probably distinct and different for each biological group of diseases. In addition, these entities are apparently present in small amounts and are a minute fraction of the evolutionary and genetic adjustments between host and pathogen species.

Clinical and field studies have shown the almost universal occurrence of some degree of protein malnutrition in lower socioeconomic groups in technically underdeveloped areas of the world. It is especially serious in post-weaning and preschool children. Children developing kwashiorkor are not necessarily those receiving the poorest basic diet, but are often the ones in whom some added stress has aggravated their underlying protein malnutrition. Field studies (Béhar et al., 1958) in Guatemala indicated that excessive mortality rate among children 1 to 4 years of age was about equally accounted for by kwashiorkor, infectious diarrhea, and systemic infections. These infections were not virulent tropical diseases, but ones common to children everywhere, i.e., measles, pertussis, chicken pox, and respiratory diseases which in the United States would cause few deaths even if untreated. Experimental attempts to demonstrate the direct interaction of protein deficiency and infection in humans have failed or have been inconclusive presumably due to the relatively mild protein malnutrition in the subjects studied (Scrimshaw et al., 1968).

To study the relation of nutritional deficiency to antibody production, Balch (1950) measured the response to a single injection of diptheria toxoid in two groups of Shick negative adults; one group of 25 were poorly nourished patients with hypoproteinemia, progressive weight loss and other signs of the terminal stages of chronic debilitating disease, the second

group consisted of well-nourished healthy adults. The nutritionally depleted patients produced large quantities of antibody similar to or greater than the healthy controls. However, total serum proteins for the majority of the depleted patients ranged from 4.1 to 6.7 g/100 ml serum with 3 patients above this level while the well-nourished group ranged from 6.3 to 7.7 g/100 ml serum. Serum albumin ranged from 1.5 to 3.9 g/100 ml and globulin from 1.8 to 5.6 g/100 ml serum in the depleted groups. In humans the normal range for total serum protein is 5.7 to 8.0 g/100 ml serum, for serum albumin, 2.8 to 4.5 g/100 ml and for serum globulin, 3.0 to 3.5 g/100 ml serum (White et al., 1964). Therefore, the depleted subjects in Balch's study appeared to be somewhat deficient in total serum protein and serum albumin, but probably not in globulins.

Effects of quantity of dietary protein

The apparent interrelationship between protein malnutrition and infection has prompted investigation of the relationship between the immune response and the quantity and quality of protein in the diet.

Cannon (1942) theorized that since antibodies were assumed to be globulins, antibody production must be influenced by the same conditions which determined globulin production, namely protein intake. Rabbits made hypoproteinemic by feeding a low protein diet exhibited a lessened capacity to produce agglutinins against bacterial antigens when compared with rabbits

maintained on a high protein diet (Cannon et al., 1943). Further studies have shown that circulating antibody titers were depressed in prolonged protein depletion (Gemeroy and Koffler, 1949; Glabais, 1946; Kenney et al., 1965).

Wissler et al. (1946) demonstrated that repletion of hypoproteinemic rats with adequate amounts of high quality protein, 9% protein from lactalbumin, 20% protein from dehydrated beef, or 20% protein from an enzymatic hydrolysate of casein, led to a markedly increased output of hemolysin to sheep erythrocytes. Protein-depleted rats repleted with 18% egg protein exhibited higher hemolysin titers than animals fed diets containing 9% egg protein (Glabais, 1946). Kenney et al. (1965) reported that hemolysin titers to sheep erythrocytes were less in protein-depleted rats than in animals on stock ration and, in addition, an 18% egg protein diet tended to produce higher antibody titers than did 9% egg protein. However, 18% casein diets depressed hemolysin titers in rats and also lowered titers of the naturally-occurring immunoglobulin, properdin, when compared with 9% casein diets.

Wissler (1947a, b) noted that agglutinin titers were reduced in both protein-depleted rabbits and rats compared with control animals fed 15% protein rations. In another study, the antibody response to beef serum was higher in adult rabbits repleted with 24% casein than in protein-depleted rabbits (Gemeroy and Koffler, 1949). Some evidence of impaired

agglutinin production was reported in rats fed 3% or 6% casein diets, while animals on diets containing 8%, 12% and 16% casein produced agglutinins as well as those fed a 16% protein stock diet (Miles, 1951). Protein-free or 3% casein diets resulted in decreased OH-agglutinin and complement-fixing antibody titers in rats while animals fed 20% casein diets or stock ration produced increased antibody titers to typhoid vaccine (Moroz, 1968).

Conversely, two investigators have reported either lowered antibody titers with high levels of dietary protein or no interference with antibody formation in protein-depleted individuals. Balch (1950) observed that nutritionally depleted patients with hypoproteinemia and progressive weight loss could produce antibody to a single injection of diptheria toxoid as well as or better than healthy controls. Healthy men in another study (Hodges et al., 1962) produced less antibody to tetanus and typhoid antigens as the quantity of egg yolk protein was increased in their diets.

Effects of protein quality on antibody production

The quality of dietary protein is another factor affecting antibody production and the immune response.

Antibody-producing mechanisms of protein-depleted rats were restored to nearly normal levels by repletion with high quality protein, for example, 9% lactalbumin protein, 20% beef protein, and 20% protein from an enzymatic hydrolysate of casein

(Wissler et al., 1946). Guggenheim and Buechler (1948) noted in rats injected intraperitoneally with Salmonella typhi murium that diets of 9% egg or meat proteins elicited bactericidal responses and phagocytic activity in the peritoneal fluid roughly equal to that elicited by 18% casein diets; 9% soybean protein diets produced a diminution of bactericidal response similar to that of 9% casein diets; 9% maize protein caused a significant decrease in both bactericidal response and phagocytic response similar to that produced by 3% casein; and 9% peanut protein diets resulted in almost complete breakdown of phagocytic activity whereas the bactericidal activity was similar to that produced by 6% casein diets.

Antibody responses to tetanus and typhoid antigens were poor in human subjects who ate a low protein diet of skimmed milk solids supplying 20 g of protein per day when compared to subjects who consumed diets supplying 1 g or 2 g of protein per kg body weight per day as either skimmed milk solids only or a mixture of skimmed milk solids plus egg yolk proteins (Hodges et al., 1962). However, although egg yolk proteins at the level of 20 g per day did not elicit poor immune responses in subjects, it was inferior to a mixture of egg plus skimmed milk proteins in supporting antibody responses when proteins were fed daily at 1 or 2 g per kg body weight.

Studies have shown that dietary regimens which may be the most favorable for growth, reproduction, or maintenance of

nitrogen equilibrium may not necessarily be the best for supporting immune response. Kenney (1963) demonstrated that adult rats repleted with casein and wheat gluten exhibited lower antibody titers than those fed soy alpha protein, although casein gave the most rapid weight gain. Supplementation of wheat gluten with lysine and soy protein with methionine produced an increase in weight gain and hepatic nitrogen; however, addition of lysine to wheat gluten did not significantly increase mean antibody titer while methionine supplementation of soy protein actually lowered antibody titers. Hemolysin titers of rats were higher when corn protein alone was fed than when corn was supplemented with fish, although the supplemented corn gave greater gains in weight (Smith and Kenney, 1969).

Ryan (1965) observed that daily injections of 40 mg phenylalanine per 100 g body weight inhibited antibody production and allowed tolerance of skin grafts in rats. However, daily injections of tyrosine, histidine, glutamate, asparate, and methionine in the same amounts produced no effect. Deficiencies of phenylalanine and tryptophan in rats reduced antibody responses to synthetic and natural antigens, whereas methionine excess or deficiency did not alter antibody responses (Gershoff et al., 1968). Gill and Gershoff (1967) noted that although the primary response of Cebus monkeys to a synthetic polypeptide antigen was resistant to either methionine deficiency or excess, the early secondary response to this antigen was

depressed by excess methionine. The late secondary response was enhanced in monkeys when they were returned to a normal diet after previous treatment with a diet containing excess methionine plus ethionine. Thus for antibody production the relative proportions of various amino acids in the diet are as important a factor as their total amount.

Dietary protein and turnover of serum proteins and antibodies

The literature contains a number of reports relating dietary protein to plasma protein turnover; however, little has been reported concerning the effect of dietary protein on antibody metabolism and turnover. Although the studies mentioned here will relate dietary protein to turnover of serum protein, globulin or gamma globulin in particular, the results may apply indirectly to antibody turnover.

Several investigators have shown that protein-depleted animals incorporated a larger proportion of an injected dose of radioactive tracer into plasma proteins than did normal animals (Garrow, 1959; Wannemacher, Jr., 1961). This enhanced uptake of radioactive tracer in serum proteins has been interpreted to indicate early changes in protein metabolism associated with protein depletion (Allison et al., 1958). Protein-depleted rats incorporated a greater proportion of injected ^{35}S-labeled methionine into serum proteins than into muscle and skin (Waterlow, 1959). Waterlow and Stephen (1966) also found that protein-depleted rats retained relatively more radioactivity in

the internal organs and less in the carcass than normal rats after an injection of L-^{14}C-lysine.

Dietary protein deprivation has reduced the catabolic rate and increased the half-life of serum albumin. Serum gamma globulin metabolism, on the other hand, was not altered significantly under the same conditions of protein depletion (Freeman and Gordon, 1964; Hoffenberg et al., 1966). The half-life of serum albumin of rats was decreased and its replacement rate increased as dietary protein was raised from 0% to 5% to 40% casein. No significant differences in the half-lives or replacement rates for α_1-, α_2-, β-, or γ-globulins were observed, however, when the diet was inadequate, adequate, or excessive in protein (Jeffay and Winzler, 1958b).

Total body albumin, rate of albumin synthesis, and fractional catabolic rate of albumin were generally significantly reduced below normal values in children with kwashiorkor or protein malnutrition (Cohen and Hansen, 1962). In contrast, the metabolism of gamma globulin was relatively unaffected by the nutritional status of the individual. When cases of kwashiorkor were complicated by infection, however, the rate of gamma globulin synthesis was three times higher than that observed in uninfected children with kwashiorkor.

Increasing the dietary protein has decreased the half-life and increased the replacement rate of plasma proteins in rats (Steinbock and Tarver, 1954; Solomon and Tarver, 1952). Plasma protein turnover in dogs was accelerated by increasing protein

intake from 0 to 2.0 g per kg body weight; however, no further acceleration was observed with protein increments up to 6.0 g per kg body weight. The more rapid plasma protein turnover rate in dogs receiving adequate dietary protein was reflected by changes in the concentrations of both the albumin and globulin fractions (Yuile et al., 1959). Muramatsu et al. (1963) observed in rats also that no further appreciable increases in plasma protein turnover rate occurred by increasing the level of dietary protein above 25 or 40%.

Dietary Protein and Splenic and Hepatic Constituents

The liver is extremely sensitive to dietary changes and has been studied extensively with respect to dietary protein. Since it is a major organ participating in protein metabolism, the behavior of liver cells to protein intake probably represents adaptation of the cellular machinery for protein synthesis and catabolism to the amino acid supply. Thus information on changes in hepatic RNA and DNA content may elucidate the mechanism of interaction between nutrition and resistance to infection.

The spleen is an important site of antibody production, hence, changes in splenic RNA and DNA content as a function of dietary protein may be reflected in antibody production. Piedad (1968) found that protein-depleted rats had lower total spleen cell counts and fewer nucleated cells per spleen than control animals fed a stock ration containing about 25% protein. Total

spleen cell count of immunized stock-fed rats was higher than that of non-immune rats, but the percentage of nucleated cells andorgan weight were not different. These data suggested that the number of splenic nucleated cells, hence splenic DNA, in rats was unstable and varied with changes in dietary protein and during the immune response.

Dietary protein and nucleic acids

Nucleic acid metabolism in the body has been affected by dietary proteins (Kosterlitz, 1947; Munro et al., 1953; Wikramanayake et al., 1953; Munro and Clark, 1960; Munro et al., 1965). Since protein synthesis and hence antibody formation are dependent on nucleic acid metabolism, dietary protein may influence the immune response indirectly through its effect on nucleic acid metabolism.

The influence of dietary protein on hepatic nucleic acid in particular has been studied extensively. Protein depletion caused a decrease in hepatic RNA, but not in DNA content (Thomson et al., 1953; Allison et al., 1961; Munro et al., 1953; Mandel et al., 1966). Decreasing the quality or quantity of dietary protein has also lowered RNA content of the liver (Zigman and Allison, 1959). Allison et al. (1961) found that the RNA and protein content of rat liver increased as the amount of dietary casein was raised from 0 to 50%. The hepatic RNA content relative to a unit weight of liver was greatest in rats fed egg protein, lowest in those fed cottonseed flour and wheat

gluten, and intermediate in those fed casein.

Hepatic DNA was found to be essentially independent of the quality or quantity of dietary protein (Allison et al., 1962; Allison et al., 1961). These studies and others indicated that, in general, DNA per liver cell was the least variable constituent of the liver during variations in dietary protein intake. On the other hand, Umaña (1965) noted that protein-deficient diets produced a significant increase in the average DNA content of the liver cell nucleus in adult rats and a significant increase in hepatic DNA concentration in both adult and weanling rats. Although hepatic DNA fell in protein-deficient rats, an apparent increase in concentration of DNA was also observed by Svoboda et al. (1966). The increased DNA concentration had been reported earlier by Williams (1961) as a result of prolonged protein depletion, but it probably reflected a more rapid loss of other cellular constituents than of DNA. Higher hepatic DNA concentration has also been reported in immunized protein-depleted rats than in immunized stock fed controls (Kenney et al., 1968).

Since RNA and DNA play important roles in protein biosynthesis and DNA is a relatively stable cell component, RNA/DNA ratios have commonly been used to assess the nutriture of an organism with respect to protein; and this, in turn, reflects the nutritive value of dietary proteins. Allison et al. (1962) obtained the highest hepatic RNA/DNA ratios with egg protein while the lowest ratios were obtained with cottonseed flour and

wheat gluten. In protein-depleted adult dogs, RNA/DNA ratios of the liver were decreased when compared with well-fed controls (Wannemacher, Jr. et al., 1963).

Banks et al. (1964) observed that hepatic RNA/DNA ratios in weanling rats increased with both an increase in nitrogen intake and an improvement in the nutritive value of some dietary proteins, but not when wheat gluten was supplemented with lysine. They suggested that free lysine was absorbed and transported in such a way that the improved growth of the rats when fed supplemented wheat gluten was associated primarily with growth of tissue such as muscle rather than liver. Lowered RNA/DNA ratios were found in protein-depleted, immunized adult rats compared with immunized animals fed a stock ration (Kenney et al., 1968).

The involvement of nucleic acids in splenic antibody production has been demonstrated by Cohen et al. (1965). Spleen cells from nonimmunized mice were converted to antibody-forming cells in tissue cultures by an extract containing RNA from spleens of immunized mice.

Lazda et al. (1968a, b) observed that the primary response of rats to flagellar antigens in vivo was characterized by an increased rate of incorporation of newly formed nucleotides into splenic RNA within 24 hours after immunization. The enhanced incorporation continued for about 48 hours and subsided to normal in 5 days. The RNA which was rapidly labeled and associated with ribosomes during this period had sedimentation

characteristics of both messenger RNA and ribosomal RNA. The RNA synthesized in greatest abundance during the period of most rapid synthesis, i.e., the first hour after tracer injection, had messenger-like sedimentation coefficients in the range of 6 to 18S. Stable ribosomal RNA accumulated extensively over the next 24 hours while messenger RNA accumulated relatively slowly. The base composition of the 6 to 12S RNA differed from the nucleotide composition of ribosomal and soluble RNA and resembled that of DNA in their (GMP + CMP)/(AMP + UMP) ratios. In addition, this RNA was characterized by relatively high UMP/AMP ratios. The base ratios of the messenger RNA which accumulated were different from those of the rapidly labeled but unstable 6 to 12S RNA. On the basis of these results, Lazda et al. concluded that the synthesis of at least 2 major species of messenger RNA was stimulated by immunization: one a labile species which may code for structural proteins and enzymes and another which was relatively stable and may be the message for antibody synthesis.

Little information has been reported concerning the effect of dietary protein on the spleen and its antibody-producing capacity. However, the spleens of protein-deficient rats were smaller and contained fewer cells, less RNA and more DNA per cell than those of well-fed controls (Kenney et al. 1968).

In the present study, serum agglutinins and hemolysins, changes in hepatic and splenic RNA and DNA, serum gamma globulin

concentration and specific activity of serum antibody and gamma globulin as related to the immune response were investigated in rats fed several levels of egg protein.

METHODS AND PROCEDURE

General Plan

This study was undertaken to evaluate diets containing different levels of high quality dietary protein on the basis of hepatic RNA and DNA, splenic RNA and DNA, weight gain, immune response, and the rates of turnover of immunoglobulins.

The investigation consisted of three consecutive experiments -- I, II, and III. Adult male rats of the Wistar strain, used in all the experiments, were born and raised in the stock colony of the Iowa State University Food and Nutrition laboratory. The animals weighed between 459 and 516 g and were 3 to 4 months old. Rats were depleted for 3 to 6 weeks on a low protein, calorie-restricted diet and repleted for approximately one week on diets that varied in quantity of egg proteins. Control groups were fed either a stock ration or a depletion diet throughout the experiment.

The animals were immunized by injecting sheep red blood cells either six days prior to autopsy or on the first or third day of repletion in which case they were killed five or six days later. When radioactive lysine was employed, it was administered five days after immunization and 3 or 24 hours prior to killing the animal.

Blood was collected 3 and/or 24 hours after injection of the tracer and the serum isolated and frozen for analyses at a

later date. Livers and spleens were removed, weighed, and frozen for later analyses also.

Table 1 gives a summary of the experimental plan.

Experiment I

The first experiment was designed to determine if restricted consumption for a limited time (L) and ad libitum or unlimited consumption (U) of a low protein diet (O) produced different effects on composition of weight loss and on the immune response of rats injected with sheep red blood cells (I). Groups fed a stock ration (S) served as controls.

Two groups of rats, O-LI and O-L, containing 9 and 5 rats, respectively, were depleted for 3 weeks in the following manner:

a) First week - fed a nitrogen-low diet ad libitum
b) Second week - fed the nitrogen-low diet restricted to half the average daily intake of the last four days of the first week. The daily diet was divided into two equal portions for a morning and an afternoon feeding.
c) Third week - fed the nitrogen-low diet further reduced by the same percentage as body weight loss during the second week of depletion; i.e., if a 10% decrease in body weight occurred during the second week of depletion, then the daily intake of the nitrogen-low diet was further reduced by 10%, for example, from 10 g/day to 9 g/day.

Table 1. Summary of experimental plan

Experiment	Group[a]	No. of rats per group[b]	Length of depletion	Length of repletion	Interval of tracer incorporation
			days	days	hours
I	O-LI	9	21		
	O-L	5	21		
	S-UIa	5			
	S-Ua	5			
	O-UI	9	38[c]		
	O-U	5	38[c]		
	S-UIb	5			
	S-Ub	5			

[a]Symbols used to denote the following:
O = low-protein diet
L = restricted or limited daily food intake
I = immunized
S = stock diet
U = unlimited daily food intake
a = kept on experiment for 21 days
b = kept on experiment for an average of 38 days
S-A-24 = stock diet for all of experiment with 24 hours of tracer incorporation

In Experiment II and III: first number represents the % egg protein fed during repletion, second number stands for the number of days repleted, and the third number is the number of hours of tracer incorporation, e.g., 18-6-24 = repleted with 18% egg protein for 6 days with 24 hours of tracer incorporation.
0-6-24-II and 0-6-24-III = groups repleted with low protein diet for 6 days with 24 hours of tracer incorporation in Experiment II and III, respectively.

[b]Rats of Wistar strain in all experiments from stock colony of Department of Food and Nutrition, Iowa State University.

[c]Average period of depletion, range was 28 days to 42 days.

Table 1 (Continued)

Experiment	Group[a]	No. of rats per group[b]	Length of depletion days	Length of repletion days	Interval of tracer incorporation hours
II	0-6-24-II	9	21	6	24
	18-6-24	10	21	6	24
	18-9-24	9	21	9	24
	S-A-24	11			24
III	0-5-3	10	26	5	3
	3-5-3	10	"	"	"
	6-5-3	10	"	"	"
	9-5-3	10	"	"	"
	0-6-24-III	10	26	6	24
	3-6-24	10	"	"	"
	6-6-24	10	"	"	"
	9-6-24	10	"	"	"

Groups O-UI and O-U with 9 and 5 rats, respectively, were fed the nitrogen-low diet ad libitum for four to six weeks. These animals were matched with those of groups O-LI and O-L for initial weight and then depleted to the same final weight attained by their partners in groups O-LI and O-L, regardless of the time required to do so.

Groups S-UIa and S-Ua with 5 rats each were fed the stock diet ad libitum for 3 weeks, while groups S-UIb and S-Ub were fed similarly for the same time periods as rats in groups O-UI and O-U.

Six days before autopsy, the rats in the following groups were injected with sheep red blood cells (Srbc) as antigen: O-LI, S-UIa, O-UI, and S-UIb. Rats were sacrificed at the end of their respective depletion or maintenance periods, either at the end of 3 weeks or after an average of 38 days.

The liver, spleen, adrenals, kidneys, and testes were removed and weighed. The liver and spleen were wrapped individually in aluminum foil, frozen in liquid nitrogen, and stored at -20° C.

Experiment II

Rats obtained from the stock colony were depleted by feeding a nitrogen-low diet for 3 weeks in a manner identical to that used for groups O-LI and O-L in Experiment I. Then the animals were divided into 3 groups and either repleted with a diet containing 18% egg protein (18-6-24 or 18-9-24) for 6 or

9 days or given the depletion diet ad libitum (0-6-24-II) for 6 days. A fourth group (S-A-24), fed the stock diet ad libitum throughout the experimental period, served as the control.

Rats in groups 18-6-24 and 0-6-24-II were immunized on the first day of repletion while those in group 18-9-24 were immunized 3 days after repletion began. Animals in group S-A-24, which had not been depleted, were immunized 6 days before killing.

Five days after immunization and 24 hours before autopsy, all the rats were injected intravenously with a 0.5 ml dose of L-lysine-^{14}C solution containing 10 μc. Blood was withdrawn from the rats three hours and 24 hours after tracer administration. The animals were sacrificed 24 hours after the radioisotope injection.

Experiment III

In this study rats from the stock colony were depleted by feeding the nitrogen-low diet in the same manner as in Experiments I and II except that the third week of depletion was extended to a 12-day period due to a delay in receiving the egg protein used in the repletion diets. The adjusted food intake for the third week of depletion was maintained throughout the 12-day period.

Starting on the 27th experimental day the animals were repleted for 5 or 6 days with a diet containing 3% egg protein (3-5-3 and 3-6-24), or 6% egg (6-5-3 and 6-6-24), or 9% egg

(9-5-3 and 9-6-24), or the depletion diet ad libitum (0-5-3 and 0-6-24-III). All rats were immunized as before with 2% Srbc on the first day of repletion, and radioactive lysine was injected on the fifth day of repletion. Three hours after radioisotope administration, groups 0-5-3, 3-5-3, 6-5-3, and 9-5-3 were sacrificed; the time of autopsy, therefore, was 5 days after immunization. Groups 0-6-24-III, 3-6-24, 6-6-24, and 9-6-24 were autopsied 24 hours after tracer injection, i.e., 6 days following immunization.

Care of animals

The rats were housed in individual wire-meshed cages on racks in an air-conditioned room at 24° to 26° C. for the major portion of the experimental period. The rats received distilled water ad libitum. Food was given ad libitum except for the two weeks of restricted feeding during the depletion period when the calculated daily food intake was divided equally into a morning and afternoon feeding.

The rats were weighed at least 3 times a week and food intake data were recorded.

Cages and racks were changed each week and papers beneath the cages were changed daily. Food jars were replaced every other day, and the uneaten food discarded. Daily vitamin supplements were given in separate containers to which 2 drops of cod-liver oil and 2 drops of alpha-tocopherol solution were added just before feeding.

Rats were transferred to a radioisotope laboratory where the isotope was administered and the animals were then placed in wire-meshed metabolic cages. Each cage was equipped with a funnel leading from the bottom of the cage to an erlenmeyer flask containing 5 ml of 5N HCl in which all excreta were collected.

Rats which were sacrificed 3 hours after tracer administration were fasted for this 3 hour interval while animals killed 24 hours after tracer injection were not fasted.

Composition and preparation of the diets

The composition of the diets is given in Table 2. The hexane extracted whole egg which was used as the protein source for the experimental repletion diets was analyzed for nitrogen.

Constant amounts of fiber, fat, and salts were incorporated into all the diets. The quantity of the protein source in each diet was varied at the expense of the starch content of the diet.

The diets were mixed in quantities of about 10 kilograms at a time. The prepared diets were stored in polyethylene containers at 4° C. until used. The composition of the stock diet is given in Table 3.

Table 4 presents the composition of the vitamin supplement given daily to all rats fed the purified diets. Animals fed the stock diet also received supplements of 5 g beef three times, 10 g carrots twice, and 10 g cabbage once each week.

Table 2. Composition, protein source, and nitrogen content of experimental diets

Ingredients	Depletion diet	Repletion egg diets			
	O-LI, O-L, O-UI, O-U, 0-6-24-II, 0-5-3, 0-6-24-III	3-5-3 3-6-24	6-5-3 6-6-24	9-5-3 9-6-24	18-6-24 18-9-24
	g/100 g diet				
Cornstarch[a]	84.00	79.64	75.27	70.91	57.82
Fat[b]	10.00	10.00	10.00	10.00	10.00
Hawk Oser salt mix[c]	4.00	4.00	4.00	4.00	4.00
Non-nutritive fiber[c]	2.00	2.00	2.00	2.00	2.00
Egg[d]		4.36	8.73	13.09	26.18
Protein source:					
% total protein (calculated values)[e]		3	6	9	18
% nitrogen (calculated values)[e]		0.48	0.96	1.44	2.88

[a]Experiment I - Argo, Best Foods Co.
Experiments II and III - General Biochemicals Inc., Chagrin Falls, Ohio.

[b]Crisco, Procter and Gamble, Cincinnati, Ohio.

[c]General Biochemicals Inc.

[d]Hexane extracted whole egg: General Biochemicals Inc.

[e]Calculated from previous analysis where hexane extracted whole egg contained 11% nitrogen or 68.75% protein, using the conversion factor 6.25.

Table 3. Composition of stock diet

Ingredients	%
Corn meal[a]	48.3
Linseed meal[b]	13.8
Wheat germ[c]	8.6
Yeast (unirradiated)[a]	8.2
Casein (crude)[d]	4.3
Alfalfa meal[e]	1.7
NaCl (iodized salt)[f]	0.4
$CaCO_3$[g] and trace elements[h]	0.4
Yeast (irradiated)[i] and calcium pantothenate	0.4
Dried skimmed milk[j]	10.2
Cottonseed oil[k]	3.6

[a]General Biochemicals, Chagrin Falls, Ohio.

[b]Froning and Deppe Elevator, Ames, Iowa.

[c]Gold Medal: General Mills Central Division, Minneapolis, Minnesota.

[d]The Borden Company, New York, New York.

[e]National Alfalfa Dehydrating and Milling Co., Kansas City, Missouri.

[f]Hy-Vee, Ames, Iowa.

[g]Matheson, Coleman and Bell, Norwood, Ohio.

[h]Trace elements:

	g		g
KI	0.400	$CuSO_4$	2.036
$MnSO_4$	1.580	$CaCO_3$ to make	1000
$K_2Al_2(SO_4)_4$	0.490		

[i]Six g Ca pantothenate added to each kg of irradiated yeast. Brewer's yeast irradiated in laboratory.

[j]Des Moines Coop Dairy, Des Moines, Iowa.

[k]Wesson oil, Wesson Sales Co., Fullerton, California.

Table 4. Vitamin mix composition given daily to rats

Components	Amount
	mg/rat/day
Thiamine hydrochloride[a]	0.040
Riboflavin	.060
Pyridoxine hydrochloride	.040
Calcium pantothenate	.100
Nicotinic acid	.500
Folic acid	.008
Biotin	.001
Vitamin B_{12}	.00075
Ascorbic acid	1.0
Choline chloride	5.0
Inositol	10.0
Para-amino benzoic acid	10.0
Cornstarch	473.2
dl-α-tocopherol	0.75[b]
Wesson oil	49.25[b]
Cod liver oil[c]	50.00[b]

[a]All crystalline vitamins purchased from General Biochemicals, Inc., Chagrin Falls, Ohio.

[b]Medicine droppers were selected and calibrated so that 2 drops would provide approximately 50 mg of Wesson oil in which the dl-α-tocopherol was dissolved and 50 mg of cod liver oil.

[c]Squibb Laboratories, Detroit, Michigan.

Immunization

A 2% suspension of sheep red blood cells in saline was used as the source of antigen throughout the entire study. One ml of antigen suspension was injected through a lateral caudal vein with a 23 or 24 gauge needle after the rat was lightly anesthetized with ether. Prior to use, the cells[1], suspended in Alsever's solution, were washed with isotonic sodium chloride solution (0.85%) until a clear wash was obtained. The washing procedure consisted of gently mixing the cells in the saline solution in a centrifuge tube and then centrifuging the suspension for 5 minutes at a speed of 2500 rpm. Following the last washing, the cell suspension was centrifuged for exactly eight minutes. The washed cells were then diluted 1:50 by volume with 0.85% NaCl solution, giving a final 2% suspension.

Radioisotope administration

Animals in Experiment II and III were injected intravenously with L-lysine-^{14}C solution five days after immunization with Srbc. The L-lysine-^{14}C solution was prepared by dissolving 0.5 mc of freeze-dried L-lysine-^{14}C (U) monohydrochloride[2,3] in 25 ml of sodium chloride solution (0.85%). A dose of 10 µc, contained in 0.5 ml of solution, was introduced through a lateral caudal vein with a 25 gauge needle after the rat was

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[1]Baltimore Biological Laboratories, Weschester, Pa.

[2]Nuclear Chicago, Chicago, Ill.

[3]Specific activity: 64.2 µc/mg.

lightly anesthetized with ether. A preliminary experiment indicated that serum radioactivity reached a peak about 2 to 4 hours after tracer injection and declined thereafter. Thus 3-hour and 24-hour blood samples were utilized to determine changes in radioactivity of serum proteins with the passage of time.

In Experiment II, three hours after radioisotope administration, one ml of blood was withdrawn from the tail of rats lightly anesthetized with ether. Blood was also collected 24 hours after tracer injection when the rats were sacrificed.

In Experiment III, groups 0-5-3, 3-5-3, 6-5-3, and 9-5-3 were sacrificed 3 hours after radioisotope administration while groups 0-6-24-III, 3-6-24, 6-6-24, and 9-6-24 were killed 24 hours after injection of tracer.

Autopsy procedure

At the time of autopsy rats were anesthetized by injecting 1.8 to 2.0 ml of sodium pentobarbital solution[1] intraperitoneally. After the animals had lost their reflexes, a midline incision was made up to the diaphragm, exposing the abdominal cavity. As much blood as possible was then withdrawn from the abdominal aorta using a sterile 20 gauge needle on a 20 ml syringe. Whenever only small amounts of blood could be obtained from the artery, the thoracic cavity was opened and blood was

[1] 50 mg/ml Abbott Laboratories, North Chicago, Illinois.

collected from the heart. The blood was poured into a centrifuge tube and allowed to clot overnight in a refrigerator. The following morning the blood was centrifuged for 15 minutes at 2000 rpm. The resultant serum was then transferred to another centrifuge tube and recentrifuged for 15 minutes more at 2000 rpm. The sera were stored in glass vials at -20° C. until used for analyses.

The liver was removed, blotted on absorbent paper to remove excess blood, weighed, wrapped in aluminum foil and frozen in liquid nitrogen.

The spleen was then excised, blotted on absorbent paper, weighed, wrapped in aluminum foil and frozen in liquid nitrogen. In Experiment I the kidneys, adrenal glands, and testes were also removed and weighed, but were not kept for any further analyses.

The quantity of abdominal fat present was noted using a rating of 1, 2, 3, or 4 which indicated a progressive increase in size of fat pads. The lungs were removed and examined and any abnormalities recorded.

Immunochemical Measurements

Titration of antibodies

Serum agglutinins and hemolysins were titrated by using a modified semi-micro method described by Smith (1966). The

following special microtiter[1] equipment was utilized: 1) plates containing 96 cups or depressions, 2) metal loops designed to contain 0.025 ml of serum and 3) metal tipped plastic dropper pipetter calibrated to deliver 0.025 ml. Duplicate samples were analyzed either on the same day or one day later. Samples were reanalyzed whenever titers of duplicates did not agree within one dilution.

Agglutinin The same procedure for determination of serum agglutinins was used throughout the study. Two drops of buffer (Pillimer et al., 1956) solution of pH 7.4 were added to the first depression of each row of the microtiter plate. One loop of serum, 0.025 ml, was then added to the buffer and mixed with it by twirling the loop in the depression. The diluted serum was incubated for 20 minutes at 56° C. to inactivate the complement. After incubation one drop of buffer was added to each of the remaining cups and serial dilutions were made of each serum sample from the original 1:3 dilution. One drop of 1.5% Srbc was added to each cup and the suspension allowed to sit for 1 1/2 hours at room temperature. The highest dilution of serum showing definite agglutination was taken as the end point and recorded. The sheep red blood cells used were washed as described in the immunization procedure, but the cells were resuspended in buffer for the final 1.5% solution.

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[1]Cooke Engineering Co., Alexandria, Virginia.

Hemolysin Prior to the titration of rat serum hemolysin, the concentration of guinea pig complement[1] required for the titration was determined. Lyophylized guinea pig serum was rehydrated with reconstituting fluid and a 1:10 dilution was prepared with $MgCl_2$-saline solution. One or two drops of buffer were added to the first depressions of 4 rows of the microtiter plate while the remaining depressions in the 4 rows each received one drop of buffer. One loop of the 1:10 diluted complement was added to the first depression of each of the 4 rows, thus effecting a 1:20 initial dilution of complement in 2 rows (1 and 2) and a 1:30 dilution in 2 other rows (3 and 4). Serial dilutions were then made in the 4 rows. One drop of 2% Srbc was added to each cup, followed by one drop of 0.2% rabbit antiserum containing anti-sheep red cell hemolysin[2]. The plate was tapped lightly to mix the solutions, covered with plastic film wrap and incubated in a water bath at 37° C. for 40 minutes. The cup in which half of the Srbc were lysed as determined by visual observation was taken as the endpoint. Four times the amount or concentration of complement that produced the endpoint was used for the hemolysin titrations of the rat sera. The rat serum to be analyzed was diluted 1:50 with buffer in a test tube before making serial dilutions in the plates. One

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[1]Bacto-complement; Difco Laboratories, Detroit, Michigan.

[2]Bacto-anti-sheep Hemolysin; Difco Laboratories, Detroit, Michigan.

loop of diluted rat serum was added to the first depression of each row (one serum sample for each row) and serial dilutions in buffer were made to the last depression. One drop of 2% Srbc and a drop of complement of appropriate concentration, as previously determined, were added to each cup. The plate was lightly tapped to mix the mixture in each cup, covered with plastic wrap, and incubated at 37° C. in a water bath for 40 minutes. The cup in which half of the Srbc were lysed was taken as the endpoint here also.

Chemical Analysis

RNA in liver and spleen

Livers and spleens that had been frozen in liquid nitrogen were homogenized and brought to 100 ml and 50 ml, respectively, with distilled water. A modification of the procedure described by Munro and Fleck (1966) was used for the RNA analyses on liver and spleen tissues. Both the liver and spleen were homogenized the night before the RNA analyses were performed. The homogenates were filtered through 2 layers of coarse gauze and appropriate aliquots of the filtrates were kept in covered centrifuge tubes at 0° C. in a constant temperature water-glycerol bath until analyzed the following morning. Triplicate samples of each homogenate containing 0.11 to 0.15 g of liver or 0.05 to 0.07 g spleen were analyzed simultaneously. Before analysis these samples of the filtrate were diluted to 5 ml with cold distilled water.

The nucleic acids and proteins were precipitated with 2.5 ml of 0.6 N cold perchloric acid (PCA) and the mixture allowed to stand for 10 minutes at 0° C. After centrifuging for 10 minutes at about 2300 rpm in the cold, the supernatant fraction was discarded and the precipitate in the inverted tube drained briefly onto filter paper. The precipitate was then washed twice with cold 0.2 N PCA and drained briefly after each centrifugation.

Hydrolysis of RNA was accomplished by thoroughly mixing the precipitate with 0.3 N KOH and incubating the mixture in a 37° C. water bath for one hour. After incubation the mixture was cooled to about 0° C. and the protein and DNA precipitated with cold 1.2 N PCA and separated by centrifuging in the cold. The supernatant which contained the hydrolyzed RNA was transferred to 50 ml or 25 ml flasks in the liver and spleen analyses, respectively. The precipitate was washed twice with 0.2 N PCA and the washings were added to the volumetric flask. Enough 0.6 N PCA was added to the flask to produce a final solution of ribonucleotides in 0.1 N PCA after the contents of the flask were brought to volume with distilled water.

A measure of RNA content was obtained from UV absorption at 310, 280, and 260 mμ[1]. According to Munro and Fleck (1966) an extinction of 1.000 at 260 mμ is equivalent to 32 μg RNA/ml

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[1]Gilford spectrophotometer.

in the case of rat liver. This value was used in the calculations of both liver and spleen RNA content. The ratio, 260-310/280-310, was used to check for possible protein contamination in the liver samples. A sample with a ratio below 1.34 was considered to be contaminated and its optical density reading was disregarded in the final calculation of the RNA content of the particular liver tissue (Munro and Fleck, 1966).

DNA in liver and spleen

DNA content of liver and spleen was measured by using the method described by Ceriotti (1952) with a modification as suggested by Keck (1956). The precipitate, obtained on acidifying the alkaline digest in the RNA analysis, was mixed well with 5 ml of 0.3 N KOH in the liver analyses or 7 ml in the spleen analyses. The mixture was allowed to stand overnight in the refrigerator to obtain the DNA in soluble form for analysis the following day. The alkaline DNA solution was centrifuged and the supernatant transferred to 25 ml or 50 ml flasks in the liver and spleen analyses, respectively. The remaining undissolved precipitate from liver was washed with 3.5 ml of 0.3 N KOH, that from spleen was washed with 5 ml. The supernatant was transferred to the respective volumetric flask along with an additional 5 ml of 0.3 N KOH in the spleen analyses. The DNA solution was brought to volume with distilled water making a final solution of DNA in 0.1 N KOH.

Two ml of the DNA solution to be tested was mixed well with 1 ml of 0.04% indole and 1 ml of 2.5 N HCl in a screw-capped culture tube. The loosely capped tube was placed in a boiling water bath for 20 minutes. After cooling the tube in cold water, the solution was extracted three times with 4 ml of chloroform each time and centrifuged to give a clear water phase. The optical density of the aqueous phase containing the DNA was read at 490 mμ[1]. The sample readings were corrected by subtracting the readings of blanks which had been treated and read in a manner identical to that used for the samples. Sample DNA values were calculated from standard solutions of salmon sperm DNA[2] which had been treated similarly as the samples and read under identical conditions.

Serum gamma globulin

The method used for isolating gamma globulin was modified from the technique described by Campbell et al. (1963). Gamma globulin was separated from rat antiserum by one-third saturation with ammonium sulfate. A total of 0.5 ml of saturated ammonium sulfate solution was added dropwise to 1.0 ml serum with thorough mixing to effect a final one-third saturation. The pH of the mixture was adjusted to 7.8 with 4 drops of 0.66 N NaOH and allowed to stand for 2 1/2 hours. The mixture was stirred

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[1]Gilford spectrophotometer.

[2]Calbiochem, Los Angeles, California.

every half hour to maintain an even suspension and prevent settling and aggregation of the particles formed. The suspension was then centrifuged at room temperature for 30 minutes. The resultant supernatant was discarded and the precipitate was redissolved in 0.90 ml of isotonic sodium chloride solution (0.85%). Two additional precipitations were carried out as before with the following differences: 1) in the second precipitation 2 drops of 0.66 N NaOH were used instead of 4 drops to raise the pH of the suspension to 7.8, 2) in the third precipitation 2 drops of 0.33 N NaOH were used to raise the pH of the suspension to 7.8, and 3) the third precipitate was dissolved in 2.5 to 3.0 ml of borate-buffered saline of pH 8.4-8.5. The dissolved precipitate, containing the gamma globulin, was transferred quantitatively to a 5 ml volumetric flask and brought to volume with additional borate-buffered saline.

The protein content of the gamma globulin solution was estimated by the Biuret Method B described by Chaykin (1966). Equal volumes of Biuret reagent and gamma globulin solution were thoroughly mixed (1:1 ratio). After the mixture stood at room temperature for 30 minutes, the optical density of the solution at 540 mμ was read on a Gilford spectrophotometer. Samples were run in duplicate and their readings were corrected by subtracting readings of blanks obtained under identical conditions. Standard solutions of bovine serum albumin were run simultaneously with the samples under identical conditions and were used to calculate the protein concentration of the samples.

Isolation of antibody

The method used to isolate specific labeled serum antibodies to sheep red blood cells was modified from the technique of Talmage et al. (1954). Prior to the isolation of antibodies, 0.5 ml of antiserum was incubated in a 56° C water bath for 30 minutes to inactivate the serum complement. The sample was cooled to room temperature and 4 drops of packed sheep red blood cells were added to each sample with thorough mixing. Before using, the sheep red blood cells had been washed with isotonic sodium chloride solution (0.85%) and a 1:1 mixture of saline plus decomplemented normal rat serum was used for the final wash. The decomplemented rat serum was used to block any nonspecific protein binding sites on the Srbc and, hence, help prevent the binding of radioactive nonspecific proteins during the isolation procedure. The packed cells were obtained by centrifuging them for 8 minutes and removing the last wash with a disposable pipette. The mixture of antiserum and Srbc was incubated for 40 minutes in a 37° C water bath and mixed after 20 minutes. After incubation the mixture was cooled in an ice bath for 5 minutes and centrifuged at about 2200 rpm for 7 minutes. The supernatant was removed with a disposable pipette and the sheep red blood cell-antibody complex (Srbc-Ab) was washed once with a solution of 0.10 ml decomplemented normal rat sera and 0.30 ml of cold sodium chloride solution (0.85%). After centrifuging the mixture and removing the supernatant,

the Srbc-Ab complex was suspended in a solution of 0.15 ml decomplemented normal rat serum and 0.35 ml of barbital buffer. The pH of the mixture was gradually lowered to 3.0, using a microelectrode, by dropwise addition of 0.2 M citric acid with constant stirring. The mixture was kept at pH 3.0 for 20 minutes and stirred every 6 minutes. The mixture was then centrifuged for 10 minutes and the acidified supernatant, containing the eluted antibody, was transferred with a disposable pipette to a calibrated centrifuge tube. The antibody solution was immediately neutralized and brought to pH 7.5, using a microelectrode, by dropwise addition of 0.5 N NaOH, stirring constantly. After centrifuging the antibody solution, the volume of the supernatant containing the antibody was recorded. The supernatant was transferred to a glass vial and stored at -20°C. for agglutinin and radioactivity measurements later.

Radioactivity Measurements

Radioactivity measurements were made on rat sera, isolated antibody solutions, gamma globulin solutions, and solutions of excreta.

A known volume of solution was pipetted onto a disc of lens paper lying on an aluminum planchet. The sample was allowed to dry at room temperature. The planchet bearing the dry sample was counted in a gas-flow detector[1] which was used

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[1]Nuclear Chicago, Chicago, Illinois.

in combination with an automatic sample changer and decade scaler. The low background detector was operated in the Geiger region with a Micromil window and it utilized a constant flow of quenching-gas during operation. Background activity of the detector, which was determined before and after sample measurements, was used to correct sample radioactivity values.

Statistical Evaluation

The "Student's" *t*-test was used to determine differences between group means (Snedecor, 1961). Group means are reported with standard errors of the means. When the range of values within a group was broad, the median value was found and used in comparing one group with another (Hoel, 1966).

RESULTS

This study was conducted in a series of three investigations: Experiments I, II, and III. Experiment I was designed to determine if limited feeding and ad libitum feeding of a low protein diet would change the composition of similar organ weight losses and the immune response of rats. In the first experiment, groups O-LI and O-L were depleted for 3 weeks by feeding a nitrogen-low diet with the food intake limited during the last 2 weeks of depletion. Groups O-UI and O-U were depleted by feeding the nitrogen-low diet ad libitum for four to six weeks and the stock diet was given continuously to groups S-UIa and S-Ua for 3 weeks and to groups S-UIb and S-Ub for four to six weeks. The following groups were injected with sheep erythrocytes (Srbc) as antigen: O-LI, O-UI, S-UIa, and S-UIb.

Experiment II was undertaken to determine the effects of protein-calorie depletion and subsequent repletion with carbohydrate and fat only or carbohydrate and fat plus protein on the immune response and turnover rate of radioactive serum components. Rats were depleted as in Experiment I by limiting the intake of a nitrogen-low diet for 2 out of 3 weeks. Then they were repleted either with an 18% egg protein diet (18-6-24 or 18-9-24) for 6 or 9 days, ad libitum, or with a 0% egg diet ad libitum (0-6-24-II) for 6 days. A fourth group (S-A-24), fed the stock diet continuously during the experimental period,

served as the control. All animals were immunized with Srbc and 24 hours prior to killing were injected intravenously with a 10 μc dose of L-lysine-^{14}C solution. Since the body weights of injected animals ranged from 366 g to 579 g, the tracer dose per kg of body weight varied from 2.73 μc/100 g to 1.72 μc/100 g body weight for rats in Experiment II (Table 12). Blood was withdrawn from the rats 3 hours after administration of the tracer and again at autopsy, 24 hours after tracer injection. The selection of these time periods was based on a preliminary study which indicated that serum radioactivity reached a peak about 3 hours after tracer injection and declined thereafter in stock-fed rats and rats repleted with 9% egg protein.

The third experiment was designed to evaluate diets containing different levels of egg protein on the bases of hepatic and splenic RNA and DNA and on weight gain. Immune response and rates of turnover of immunoglobulins were measured in the same animals. Rats were depleted as previously for 26 days by feeding a nitrogen-low diet ad libitum for the first 7 days and a restricted amount from the 8th day through the 26th day of the experiment. The third week of depletion was extended 5 days longer than in Experiment II due to a delay in receiving the egg protein utilized in the repletion diets. Following depletion the rats were repleted for 5 or 6 days with diets containing 3% egg protein (3-5-3 and 3-6-24), or 6% egg (6-5-3 and 6-6-24), or 9% egg (9-5-3 and 9-6-24) or 0% egg (0-5-3 and

Table 5. Mean initial body weight, total weight loss on depletion or weight gain during the experiment, and weights of liver, spleen, adrenal glands, kidneys and testes at autopsy - Experiment I.

Group	No. of rats	Mean initial body weight	Weight change	Liver		Spleen	
				total weight	weight/100 g body weight	total weight	weight/100 g body weight
		g	g	g	g	g	g
O-LI[a]	9	486±5*	-100±3	8.37±0.31[1]**	2.16±0.05	0.572±0.027[1]	0.15±0.02
O-L[a]	5	478±7	-101±7	8.51±0.52[1]	2.26±0.29	0.546±0.008[1]	0.14±0.01
O-UI[b]	9	490±5	-98±2	12.95±0.65[2]	3.30±0.14	0.572±0.036[1]	0.15±0.01
O-U[b]	5	475±5	-95±5	11.88±0.65[2]	3.13±0.17	0.517±0.038[1]	0.13±0.01
S-UIa	5	498±4	+48±5	16.56±0.73[3]	3.03±0.12	1.037±0.092[2]	0.19±0.02
S-Ua	5	484±5	+54±8	16.40±0.42[3]	3.04±0.07	1.020±0.065[2]	0.19±0.03
S-UIb	5	491±5	+78±9	15.83±0.62[3]	2.83±0.20	1.055±0.039[2]	0.21±0.01
S-Ub	5	476±4	+85±10	16.95±0.74[3]	3.02±0.21	0.817±0.031[3]	0.15±0.01

[a]Restricted depletion for 21 days.

[b]*Ad libitum* depletion for an average of 38 days.

*Mean ± S. E.

**Statistical analysis: Means of groups with the same numbered superscript in a column are not statistically different ($P < 0.05$)

Table 5 (Continued)

Group	No. of rats	Adrenal glands		Kidneys		Testes	
		Total weight g	Weight/100 g body weight g	Total weight g	Weight/100 g body weight g	Total weight g	Weight/100g body weight g
O-LI	9	0.043±0.002[1]	0.012±0.001	2.04±0.08[1,2]	0.50±0.05	3.57±0.07[1,2]	0.93±0.02
O-L	5	0.045±0.004[1,2]	0.012±0.001	2.05±0.04[2]	0.55±0.02	3.55±0.15[1,3]	0.95±0.05
O-UI	9	0.037±0.002[3]	0.009±0.002	1.85±0.05[3]	0.47±0.02	3.27±0.11[1]	0.84±0.03
O-U	5	0.034±0.003[3]	0.008±0.001	1.87±0.07[1,3]	0.49±0.02	3.51±0.23[1,3]	0.90±0.05
S-UIa	5	0.057±0.002[2]	0.010±0.001	3.07±0.07[4]	0.56±0.01	4.01±0.22[3]	0.74±0.05
S-Ua	5	0.051±0.004[2]	0.009±0.002	2.88±0.05[4]	0.54±0.01	3.82±0.11[2,3]	0.71±0.03
S-UIb	5	0.062±0.006[2]	0.011±0.001	3.13±0.11[4]	0.56±0.01	3.81±0.17[2,3]	0.68±0.04
S-Ub	5	0.049±0.002[1,2]	0.009±0.001	3.08±0.09[4]	0.55±0.01	4.10±0.36[2,3]	0.73±0.04

0-6-24-III) ad libitum. All animals were immunized with Srbc and injected with 10 μc of L-lysine-^{14}C solution. Body weights of injected rats ranged from 329 g to 464 g; hence, the tracer dose varied from 3.04 μc to 2.16 μc/100 g body weight in Experiment III. Groups 0-5-3, 3-5-3, 6-5-3, and 9-5-3 were killed 3 hours after radioisotope administration while groups 0-6-24-III, 3-6-24, 6-6-24, and 9-6-24 were sacrificed 24 hours afterward.

Rats in all the experiments were obtained from the colony maintained by the Nutrition Research laboratory at Iowa State University. They were weaned at 50 g and raised on a stock diet until placed on experimental diets.

Body weight, food intake, and nitrogen efficiency ratio

All adult male rats in this study were between 3 and 4 months old and groups averaged from 475 to 498 g in weight (Tables 5 and 6). Protein and calorie depletion for 3 weeks (O-LI and O-L) resulted in weight losses similar to those obtained by protein depletion only for 4 to 6 weeks (O-UI and O-U) and amounted to approximately 20% of the initial body weights. Rats fed the stock diet ad libitum for 3 weeks (S-UIa and S-Ua) gained approximately 10% of their initial body weight while rats fed the stock diet ad libitum for 4 to 6 weeks gained about 17% (S-UIb and S-Ub). Immunization of the rats did not appear to alter either weight gain or weight loss in Experiment I.

Table 6. Mean initial body weight, total weight loss on depletion, weight gain on repletion, food intake per day during repletion, nitrogen intake and nitrogen efficiency ratio of diets - Experiments II and III

Group	No. of rats	Mean initial body weight g	Weight loss on depletion g	Weight gain on repletion[a] g	Food intake per day during repletion[a] g	Nitrogen intake during repletion[a] g	Nitrogen efficiency ratio[b]
Experiment II							
0-6-24-II[c]	9	481±3*	104±4	6±1	18.1±0.46^{1,2}**		
18-6-24	10	480±2	111±5	50±2	17.5±0.59^{1}	2.51±0.09	20.1±0.7^{1}
18-9-24	9	482±2	101±5	61±5	17.7±0.56^{1}	4.06±0.13	14.8±0.8^{2}
S-A-24	11	481±3	(55±5)[d]		(19.3±0.66)		
Experiment III[e]							
0-5-3	10	482±4	118±3	13±2^{1}	16.6±0.60^{1}		
0-6-24-III	10	476±3	126±8				
3-5-3	10	481±3	121±5	40±2^{2}	21.1±0.48^{3}	0.51±0.01	79.2±3.0^{3}
3-6-24	10	475±4	117±2				
6-5-3	10	483±3	121±6	55±2^{3}	19.9±0.76^{2,3}	0.96±0.04	57.4±1.5^{4}
6-6-24	10	478±4	123±6				
9-5-3	10	484±3	120±5	60±2^{3}	19.4±0.66^{2}	1.40±0.05	43.2±1.4^{5}
9-6-24	10	479±5	119±7				

[a]For 5 days for groups 0-6-24-II, 18-6-24, and all groups in Experiment III and for 8 days for group 18-9-24 in Experiment II.

[b]Gain in weight/g nitrogen intake.

[c]Depleted 21 days and groups 0-6-24-II and 18-6-24 repleted 6 days and group 18-9-24 repleted 9 days.

[d]Average weight gain on stock diet for 26 days.

[e]Depleted 26 days and groups 0-5-3, 3-5-3, 6-5-3, and 9-5-3 repleted 5 days and groups 0-6-24-III, 3-6-24, 6-6-24, and 9-6-24 repleted 6 days.

*Mean ±S. E.

**Statistical analyses: Means of groups with the same numbered superscript in a column are not statistically different ($P < 0.05$).

The suitability of protein-calorie depletion over a 3-week period was established in Experiment I and therefore, adopted in the succeeding experiments. The body weight lost in Experiment II averaged 22%. However, due to the extension of the depletion period in Experiment III from 21 days to 26 days, body weight losses were approximately 25%. Animals fed the stock diet ad libitum in Experiment II (S-A-24) gained about 10% of their initial body weight during the experimental period.

In Experiments II and III food intakes and body weights were not recorded for the last experimental day after the animals were injected with radioactive lysine and placed in the isotope laboratory. Therefore, weight gains and food intakes during repletion were calculated on the basis of 8 days of refeeding for group 18-9-24 in Experiment II and on 5 days for groups 18-6-24 in Experiment II and for all groups in Experiment III.

In Experiment II rats repleted with 0% egg protein ad libitum (0-6-24-II) gained only 6 g in 5 days whereas repletion with 18% egg protein produced weight gains of 50 g after 5 days (18-6-24) and 61 g after 8 days of repletion (18-9-24). Group S-A-24, which was fed the stock diet ad libitum throughout the experiment, 26 days, gained an average of 55 g (Table 6).

Mean daily food intakes were similar for all repleted groups in Experiment II, although animals refed 0% egg protein ad libitum consumed about 0.5 g more food on the average than

rats refed with the egg protein diets. Stock-fed animals had the highest mean daily food intake of all groups; however, the stock diet had a slightly lower energy value per gram than the repletion diets.

As expected, a significantly higher nitrogen efficiency ratio (NER) was obtained when the 18% egg diet was fed for 5 days than when fed for 8 days.

In Experiment III since weight gain and daily food intake were recorded for 5 days of repletion for all groups, animal data were analyzed by combining values during refeeding into the following 4 groups: 1) 0% egg protein (0-5-3 and 0-6-24-III), 2) 3% egg protein (3-5-3 and 3-6-24), 3) 6% egg (6-5-3 and 6-6-24), and 4) 9% egg (9-5-3 and 9-6-24) ad libitum (Table 6).

Rats repleted for 5 days with 0% egg protein gained 13 g which was significantly lower than that of rats refed with egg protein diets. Repletion with 6% and 9% egg produced similar gains in body weight, 55 to 60 g, which were significantly greater than the 40 g gained by rats fed 3% egg. Groups refed with 6% or 9% egg protein regained approximately half their lost weight while animals receiving 3% egg gained about 1/3 and those refed 0% egg about 1/9 of their lost body weight.

Mean daily food intake during repletion of rats refed 0% egg protein was significantly lower than that of any other group in Experiment III. Animals refed with 6% and 9% egg had similar food intakes of 19 to 20 g which was lower than that

of rats receiving 3% egg; however, it was significantly lower than that of the group receiving 3% egg only for the group refed with 9% egg.

Nitrogen efficiency ratios decreased from 79 to 57 to 43 as the level of egg protein was increased from 3% to 6% to 9% in Experiment III and the differences among the 3 protein levels were significant. In addition, feeding 18% egg in Experiment II produced nitrogen efficiency ratios of 20 and 15 which were significantly lower than all those obtained in Experiment III. Therefore, nitrogen utilization with respect body weight gain was very efficient with 3% egg protein, but decreased significantly as the percent of egg was increased in the diet.

On the basis of the different parameters discussed thus far, raising dietary egg protein from 0 to 18% produced a concurrent increase in body weight gain but a significant decrease in nitrogen efficiency ratio.

Organ Weights in Depleted Rats

Experiment I

Mean weights of livers, spleens, adrenal glands, kidneys, and testes of rats fed a depletion or stock diet are given in Table 5. Organ weight data from stock-fed animals and animals depleted by feeding a nitrogen-low diet on a restricted or ad libitum basis served as the basis for the protocol in the two succeeding experiments.

In general, mean weights of livers, spleens, adrenals, kidneys, and testes were low in depleted groups and high in stock-fed animals. All groups of stock-fed rats had similar mean liver weights which were significantly higher than those of all the depleted groups. However, rats fed restricted amounts of the nitrogen-low diet for 3 weeks had significantly smaller livers than rats depleted ad libitum for an average of 38 days. Immunization did not affect liver weights in either the stock-fed or depleted animals. Pale livers indicative of fatty infiltration were found in groups depleted of protein ad libitum (O-UI and O-U), but not in those depleted by feeding restricted amounts of the nitrogen-low diet (O-LI and O-L).

Mean spleen weights were similar in all depleted groups, but were significantly lower than those of the stock-fed groups. Similar spleen weights were observed in the four groups fed stock diet except group S-Ub which had a lower mean spleen weight than the others. Immunized animals tended to have slightly higher mean spleen weights than their nonimmunized counterparts but the differences were not statistically significant except S-UIb.

Similar mean weights of the adrenal glands were observed in stock-fed groups; in some cases, but not all, the adrenal glands of depleted groups weighed significantly less than those of stock-fed groups. Ad libitum feeding of the nitrogen-low diet an average of 38 days (O-UI and O-U) produced significantly

lower mean adrenal weights than restricted feeding for 21 days (O-LI and O-L). Immunization did not affect the weight of the adrenals in either stock-fed or depleted groups.

Significantly larger mean kidney weights were observed in the stock-fed groups than in the depleted groups. The mean kidney weights of immune and nonimmune rats depleted by ad libitum feeding of the nitrogen-low diet were significantly lower than those of the non-immune rats depleted by restricted feeding of the same diet. Immunization did not have any effect on mean kidney weight.

Mean testes weights were lower in the depleted groups than in the stock-fed groups; however, this difference was significant in only a few comparisons. Mean weight of the testes was not affected by immunization.

When organ weights were based on 100 g body weight, the kidneys and adrenal glands were similar for the depleted and stock-fed groups while liver and spleen weights were lower in the depleted groups than in the stock animals. However, testes were larger in depleted than in stock-fed rats; hence, the testes were preferentially maintained during depletion in contrast to the other organs studied (Table 5).

Liver

Experiments II and III

In Experiment II animals repleted with 0% egg protein (0-6-24-II) had significantly lower mean liver weight and also

mean liver weight/100 g body weight than animals repleted with 18% egg protein or fed the stock diet (Table 7). Repletion with 18% egg protein for 6 or 9 days produced mean liver weights similar to each other and somewhat less than that of stock-fed rats.

In Experiment III animals repleted with 0% egg protein had significantly lower mean liver weights and liver weights/100 g body weight than rats repleted with diets containing protein. Repletion with 3%, 6% or 9% egg protein for 5 or 6 days produced similar actual liver weights as well as weight per 100 g body weight. Egg protein at the 18% level in Experiment II produced mean liver weights approximately 1 g to 2.2 g less than those of protein-repleted groups in Experiment III. Mean liver weights of all groups fed egg protein in Experiment II and III were lower than those of rats fed the stock ration in Experiment II; however, liver weights/100 g body weight were lower in stock-fed rats than in groups repleted with 3% to 9% egg protein, but similar to rats fed 18% egg protein.

Harper et al. (1953 and 1955) have shown that fatty livers of protein deficient rats were lighter in color than livers of normal rats. Pale livers, perhaps due to fatty infiltration, were found in Experiment III in rats repleted with 3% egg protein and to a lesser degree in rats repleted with 0% egg protein in Experiments II and III. This subjective observation, used as an indication of hepatic fat, suggested that the percentage of hepatic lipid was greatest in animals repleted with

Table 7. Mean weight and nucleic acids of livers of animals repleted with 0% to 18% egg protein or fed a stock ration

Experiment	Group	No. of rats	Total weight g	Weight / 100 g body weight g	No. of rats	Total RNA mg	mg RNA / g liver tissue mg
II	0-6-24-II	9	9.97±0.24*,**	2.59±0.04			
	S-A-24	11	15.43±0.50	2.86±0.06			
	18-6-24	10	11.86±0.35	2.78±0.06	7	87.6±2.9	7.38±0.42
	18-9-24	9	12.19±0.47	2.78±0.09	5	82.7±4.2	7.15±0.10
III	0-5-3	10	10.50±0.27	2.30±0.06	10	61.1±1.8	5.84±0.21
	3-5-3	10	13.25±0.50	3.32±0.12	10	73.6±2.4	5.59±0.16
	6-5-3	10	14.22±0.62	3.38±0.01	10	79.6±3.6	5.64±0.23
	9-5-3	10	13.09±0.59	3.11±0.12	9	82.3±3.4	6.44±0.29
	0-6-24-III	10	10.07±0.27	2.80±0.07	8	59.5±1.8	5.89±0.20
	3-6-24	10	13.02±0.83	3.26±0.20	8	82.9±3.7	6.69±0.29
	6-6-24	10	13.43±0.47	3.32±0.01	8	83.1±1.4	6.26±0.20
	9-6-24	10	13.99±0.64	3.38±0.16	9	92.2±5.0	6.56±0.28

*Mean ± S. E.

**Statistical analyses: Means of groups with the same numbered superscript in a horizontal row are not statistically different ($P < 0.05$). Comparisons made among all groups in Experiment II, among 5-day repleted groups in Experiment III, among 6- and 9-day repleted groups in Experiments II and III.

Total liver weight: 0-6-24-II[1], 18-6-24[2], 18-9-24[2], S-A-24[3],
0-5-3[1], 3-5-3[2], 6-5-3[2], 9-5-3[2]
{0-6-24-III[1], 18-6-24[2], 18-9-24[2,3], 3-6-24[2,3,4],
6-6-24[3,4], 9-6-24[4] (footnote continued on following page)

Table 7 (Continued)

Experiment	Group	No. of rats	Total DNA mg	mg DNA / g liver tissue mg	RNA / DNA
II	0-6-24-II	9			
	S-A-24	11			
	18-6-24	7	41.6±1.2	3.53±0.18	2.12±0.10
	18-9-24	5	41.4±2.2	3.58±0.08	2.00±0.03
	0-5-3	8	33.4±1.5	3.15±0.13	1.81±0.06
	3-5-3	8	36.2±2.3	2.69±0.18	2.10±0.12
	6-5-3	8	37.0±1.9	2.57±0.19	2.24±0.13
	9-5-3	8	38.6±0.7	3.07±0.16	2.15±0.10
	0-6-24-III	8	30.7±0.9	3.04±0.12	1.94±0.06
	3-6-24	8	35.0±1.9	2.85±0.21	2.38±0.07
	6-6-24	8	35.7±1.4	2.70±0.17	2.35±0.09
	9-6-24	8	35.5±1.2	2.53±0.13	2.69±0.12

(footnote continued from previous page)

Total liver RNA: 0-5-3[1], 3-5-3[2], 6-5-3[2,3], 9-5-3[3]
0-6-24-III[1], 18-9-24[2], 3-6-24[2], 6-6-24[2], 18-6-24[2], 9-6-24[2]

RNA/g liver: 3-5-3[1], 6-5-3[1], 0-5-3[1,2], 9-5-3[2]
0-6-24-III[1], 6-6-24[1,2], 9-6-24[1,2,3], 3-6-24[2,3,4], 18-9-24[3,4], 18-6-24[4]

Total DNA: 0-5-3[1], 3-5-3[1,2], 6-5-3[1,2], 9-5-3[2]
0-6-24-III[1], 3-6-24[1,2,3], 9-6-24[2], 6-6-24[2], 18-9-24[3,4], 18-6-24[4]

DNA/g liver: 6-5-3[1], 3-5-3[1,2], 9-5-3[1,2], 0-5-3[2]
9-6-24[1], 6-6-24[1,2], 3-6-24[1,2], 0-6-24-III[2], 18-6-24[3], 18-9-24[3]

RNA/DNA: 0-5-3[1], 3-5-3[2], 9-5-3[2], 6-5-3[2]
0-6-24-III[1], 18-9-24[1], 18-6-24[1,2], 6-6-24[2], 3-6-24[2], 9-6-24[3]

3% egg protein and with 0% egg protein.

Hepatic RNA and DNA were determined for all groups in Experiment III and for groups fed 18% egg protein in Experiment II. These parameters were expressed as amount per whole liver, per g of liver tissue, or as the ratio of RNA to DNA (Table 7).

In Experiment III after both 5 and 6 days of repletion, total hepatic RNA was significantly lower in the groups refed 0% egg (0-5-3 and 0-6-24-III) than in the egg protein-repleted groups (3-5-3, 3-6-24, 6-5-3, 6-6-24, 9-5-3, and 9-6-24). As the amount of dietary egg protein was raised, total liver RNA increased, but the only significant difference was observed between groups fed 3% egg and 9% egg protein after 5 days of refeeding but not after 6 days.

Repletion with 18% egg protein for 6 or 9 days produced values for total liver RNA which were not significantly different from those observed after 6 days of repletion with 3%, 6% or 9% egg. Slightly higher values for total liver RNA were obtained after 6 days of repletion with egg protein than after 5 days, but repletion with 0% egg protein gave similar total liver RNA values for both 5 and 6 days. For the 5 day repletion period but not the 6 day period, a regression equation showed a linear relationship between total hepatic RNA and the level of dietary egg protein. Total hepatic RNA was increased about 2.33 mg for each 1% increment of dietary egg protein up to a level of 9% egg protein.

Values for mg RNA/g liver after 5 days of repletion were significantly higher for rats fed 9% egg than for animals fed 3% or 6% egg; however, no significant difference was observed between groups repleted with 0% egg protein and those repleted with 3% and 6%. The only significant difference in mg RNA/g liver after 6 days of repletion was observed between the lowest value for rats repleted with 0% egg protein and the highest value for those refed with 3% egg. Repletion with 18% egg for 6 and 9 days produced values of mg RNA/g liver similar to each other and to repletion for 6 days with 3% egg. However, only the 6-day repletion value was significantly different from those obtained with 0%, 6%, and 9% egg protein repletion for 6 days due to the broader range of values obtained for rats repleted 9 days with 18% egg.

Livers of rats repleted 5 days with 0% egg protein had relatively low amounts of DNA, 33.4 mg; however, the total DNA was only significantly lower than that of rats fed 9% egg, 38.6 mg.

Six-day repletion with 0% egg protein was associated with significantly lower amounts of DNA, 30.7 mg, than repletion with 6% egg, 35.7 mg, and 9% egg, 35.5 mg, but not with 3% egg, 35.0 mg. No significant differences in total hepatic DNA were observed among the 3 protein levels, 3%, 6% and 9% egg, after either 5 or 6 days of repletion.

Repletion with 18% egg for 6 or 9 days produced similar amounts of DNA; these amounts were significantly higher than

those obtained by repletion with 0% egg, 6%, or 9% egg protein for 6 days. Repletion with 18% egg for 6 days but not for 9 days resulted in significantly greater amounts of hepatic DNA than 6 day repletion with 3% egg. For the 5-day but not the 6-day repletion period, a regression equation indicated that total liver DNA was related linearly to the level of dietary egg protein, 0.55 mg DNA per 1% increase of dietary egg protein.

Liver DNA concentration after 5 and 6 days of repletion was higher in groups fed 0% egg than in those fed 3%, 6%, or 9% egg protein. However, hepatic DNA concentration was significantly lower only in rats refed with 6% egg protein for 5 days and in rats given 9% egg for 6 days. Repletion for 5 or 6 days produced no significant differences in DNA concentration among groups fed 3%, 6%, or 9% egg protein. DNA concentrations were similar for groups repleted with 18% egg for 6 or 9 days and were significantly higher than those of groups repleted 6 days with 0%, 3%, 6%, or 9% egg protein.

Since the ratio, RNA/DNA has often been used to evaluate nucleic acid content of hepatic tissues and to assess nutritive value of proteins, this parameter was also estimated from the hepatic data. Hepatic RNA/DNA ratios of groups repleted for 5 or 6 days with 0% egg were significantly lower than those of all groups repleted with 3% to 9% egg protein. The RNA/DNA ratios for groups repleted 5 days with 3%, 6%, and 9% egg protein were similar. Repletion for 6 days with 3% or 6% egg

produced RNA/DNA ratios of 2.38 and 2.35, respectively, both of which were significantly lower than 2.69 obtained after 6 days on 9% egg protein. Repletion with 18% egg for 6 or 9 days produced RNA/DNA ratios which were similar to each other and to values for animals repleted for 6 days with 0% egg protein.

Spleen and Immune Response

Experiment I

In Experiment I mean spleen weights and mean agglutinin and hemolysin titers were similar for immunized groups depleted either ad libitum (O-UI) or on a restricted basis (O-LI) (Tables 5 and 8). Significantly higher values for these parameters were found in groups fed the stock diet either for 3 weeks or for an average of 38 days. Therefore, protein-calorie depletion for 3 weeks and protein depletion for an average of 38 days acted identically in reducing spleen weight and agglutinin and hemolysin responses to sheep red blood cells. The significantly higher antibody titers for stock-fed rats than for depleted rats confirm the findings of previous experiments in this laboratory that protein deficiency has a detrimental effect on the immune response in rats (Kenney et al., 1965 and 1968; Piedad, 1968; Glabais, 1946).

Table 8. Mean titers of hemolysins and agglutinins in Experiment I

Group	Agglutinin titer log	Hemolysin titer log
O-LI	$2.56 \pm 0.09^{1*,**}$	$3.38 \pm 0.08^{1,3}$
O-UI	2.57 ± 0.10^{1}	3.40 ± 0.05^{1}
S-UIa	2.98 ± 0.13^{2}	3.69 ± 0.09^{2}
S-UIb	2.95 ± 0.15^{2}	$3.65 \pm 0.12^{2,3}$

*Mean ± S. E.

**Statistical analyses: Means of groups with the same numbered superscript in a column are not statistically different ($P < 0.05$).

Experiment II

Mean spleen weight of rats repleted with the nitrogen-low diet was significantly lower than that of rats repleted with 18% egg protein or rats fed the stock diet (Table 9). Repletion with 18% egg for 6 or 9 days produced values which were similar to each other but which were significantly lower than those of stock-fed controls.

Mean agglutinin titers in Experiment II differed significantly only between stock-fed controls and animals refed 0% egg protein (Table 10).

Mean hemolysin titers for all groups in Experiment II did not differ statistically.

Splenic RNA and DNA in Experiment II were determined for groups repleted with 18% egg protein (Table 9). For rats repleted 6 days, total splenic RNA, RNA/g spleen, and total DNA were somewhat higher but not statistically different from values obtained after repletion for 9 days. Mean splenic DNA concentrations and RNA/DNA ratios were also similar.

Experiment III

In Experiment III mean weight of spleens tended to increase as dietary egg protein was raised from 0 to 6% in the 5-day repleted groups and from 0 to 9% in the 6-day repleted groups (Table 9). Rats repleted with 0% egg had significantly lower mean spleen weights than all groups of animals repleted with protein in both the 5 and 6 day repletion periods.

Table 9. Mean splenic weight and nucleic acids in spleens of animals repleted with 0% to 18% egg protein or fed a stock ration - Experiments II and III

Experiment	Group	No. of rats	Total weight	Weight / 100 g body weight	No. of rats	Total RNA	mg RNA / g spleen tissue
			g	g		mg	mg
II	0-6-24-II	9	0.602±0.028*,**	0.158±0.019			
	S-A-24	11	1.022±0.048	0.190±0.008			
	18-6-24	10	0.856±0.040	0.204±0.008	6	5.73±0.47	6.24±0.24
	18-9-24	9	0.866±0.056	0.197±0.025	6	4.61±0.29	5.96±0.27
III	0-5-3	10	0.598±0.026	0.160±0.007	8	3.02±0.17	4.90±0.15
	3-5-3	10	0.744±0.041	0.187±0.012	8	4.18±0.25	5.57±0.13
	6-5-3	10	0.857±0.038	0.204±0.009	8	4.88±0.32	5.55±0.13
	9-5-3	10	0.816±0.043	0.195±0.011	8	4.57±0.38	5.57±0.17
	0-6-24-III	10	0.616±0.032	0.170±0.010	8	3.23±0.19	5.13±0.18
	3-6-24	10	0.736±0.046	0.185±0.012	8	4.08±0.32	5.51±0.13
	6-6-24	10	0.769±0.040	0.190±0.011	8	4.53±0.42	5.75±0.27
	9-6-24	10	0.864±0.051	0.209±0.012	8	5.40±0.42	5.99±0.14

*Mean ± S. E.

**Statistical analyses: Means of groups with the same numbered superscript in a horizontal row are not statistically different ($P < 0.05$). Comparisons made among all groups in Experiment II, among 5-day repleted groups in Experiment III, and among 6- and 9-day repleted groups in Experiments II and III.

Total spleen weight: $0\text{-}6\text{-}24\text{-}II^{1}$, $18\text{-}6\text{-}24^{2}$, $18\text{-}9\text{-}24^{2}$, $S\text{-}A\text{-}24^{3}$
$0\text{-}5\text{-}3^{1}$, $3\text{-}5\text{-}3^{2}$, $9\text{-}5\text{-}3^{2}$, $6\text{-}5\text{-}3^{2}$
$0\text{-}6\text{-}24\text{-}III^{1}$, $3\text{-}6\text{-}24^{2}$, $6\text{-}6\text{-}24^{2}$, $18\text{-}6\text{-}24^{2}$, $9\text{-}6\text{-}24^{2}$, $18\text{-}9\text{-}24^{2}$

Total splenic RNA: $0\text{-}5\text{-}3^{1}$, $3\text{-}5\text{-}3^{2}$, $9\text{-}5\text{-}3^{2}$, $6\text{-}5\text{-}3^{2}$
$0\text{-}6\text{-}24\text{-}III^{1}$, $3\text{-}6\text{-}24^{2}$, $6\text{-}6\text{-}24^{2,3}$, $18\text{-}9\text{-}24^{2,3}$, $9\text{-}6\text{-}24^{3}$, $18\text{-}6\text{-}24^{3}$

(footnote continued on following page)

Table 9 (Continued)

Experiment	Group	No. of rats	Total weight	Total DNA mg	mg DNA / g spleen tissue mg	RNA / DNA
II	18-6-24	6	0.912±0.054	14.10±0.68	15.64±1.04	0.41±0.03
	18-9-24	6	0.778±0.048	12.23±0.98	15.73±0.70	0.38±0.01
III	0-5-3	8	0.616±0.027	10.08±0.91	16.28±1.10	0.31±0.02
	3-5-3	8	0.754±0.051	12.32±1.09	16.48±1.14	0.35±0.03
	6-5-3	8	0.875±0.046	13.75±1.15	15.66±0.81	0.36±0.02
	9-5-3	8	0.816±0.049	14.58±1.73	17.52±1.16	0.33±0.02
	0-6-24-III	8	0.632±0.035	11.12±0.98	17.52±1.09	0.30±0.02
	3-6-24	8	0.738±0.043	12.88±1.14	17.34±0.77	0.32±0.01
	6-6-24	8	0.781±0.049	13.73±1.29	17.54±1.20	0.34±0.02
	9-6-24	8	0.898±0.058	15.03±1.29	16.67±0.68	0.36±0.02

(footnote continued from previous page)

RNA/g spleen: 0-5-3[1], 6-5-3[2], 3-5-3[2], 9-5-3[2]
0-6-24-III[1], 3-6-24[1,3], 6-6-24[1,4], 18-9-24[3,4], 9-6-24[4], 18-6-24[4]

Total DNA: 0-5-3[1], 3-5-3[1,2], 6-5-3[2], 9-5-3[2]
0-6-24-III[1], 18-9-24[1], 3-6-24[1,2], 6-6-24[1,2], 18-6-24[2], 9-6-24[2]

DNA/g spleen: 0-5-3[1], 3-5-3[1], 6-5-3[1], 9-5-3[1]
0-6-24-III[1], 3-6-24[1], 6-6-24[1], 9-6-24[1], 18-6-24[1], 18-9-24[1]

RNA/DNA: 0-5-3[1], 3-5-3[1], 6-5-3[1], 9-5-3[1]
0-6-24-III[1], 3-6-24[1,2], 6-6-24[1,3], 9-6-24[2,3], 18-9-24[3], 18-6-24[3]

Table 10. Mean antibody titers, agglutinins and hemolysins, and gamma globulin concentrations in Experiments II and III

Group	No. of rats	Agglutinin log	Hemolysin log
Experiment II			
0-6-24-II	9	2.13±0.13*,**	2.97±0.17
18-6-24	10	2.38±0.05	3.09±0.11
18-9-24	9	2.21±0.13	3.24±0.13
S-A-24	11	2.51±0.09	3.24±0.08
Experiment III			
0-5-3	10	2.08±0.12	2.99±0.11
0-6-24-III	10	2.27±0.14	3.22±0.15
3-5-3	10	2.37±0.09	3.38±0.08
3-6-24	10	2.25±0.07	3.05±0.12
6-5-3	10	1.89±0.14	2.95±0.22
6-6-24	10	2.14±0.12	3.06±0.10
9-5-3	10	2.03±0.08	3.07±0.18
9-6-24	10	2.10±0.12	2.94±0.14

*Mean ± S. E.

**Statistical analyses: Means of groups with the same numbered superscript in a horizontal row are not statistically different ($P < 0.05$). Comparisons made among all groups in Experiment II, among 5-day repleted groups in Experiment III and among 6- and 9-day repleted groups in Experiments II and III.

Agglutinins: $0\text{-}6\text{-}24\text{-}II^{1}$, $18\text{-}9\text{-}24^{1,2}$, $18\text{-}6\text{-}24^{1,2}$, $S\text{-}A\text{-}24^{2}$
$6\text{-}5\text{-}3^{1}$, $9\text{-}5\text{-}3^{1}$, $0\text{-}5\text{-}3^{1,2}$, $3\text{-}5\text{-}3^{2}$
$9\text{-}6\text{-}24^{1}$, $6\text{-}6\text{-}24^{1,2}$, $18\text{-}9\text{-}24^{1,2}$, $3\text{-}6\text{-}24^{1,2}$
$0\text{-}6\text{-}24\text{-}III^{1,2}$, $18\text{-}6\text{-}24^{2}$

Hemolysins: $6\text{-}5\text{-}3^{1,2}$, $0\text{-}5\text{-}3^{1}$, $9\text{-}5\text{-}3^{1,2}$, $3\text{-}5\text{-}3^{2}$

mg gamma globulin/ml serum: $0\text{-}5\text{-}3^{1}$, $6\text{-}5\text{-}3^{1,2}$, $3\text{-}5\text{-}3^{2}$, $9\text{-}5\text{-}3^{2}$

Table 10 (Continued)

Group	mg gamma globulin / ml serum (mg)	Pooled data[a] mg gamma globulin / ml serum (mg)
Experiment II		
0-6-24-II		
18-6-24	6.25±0.27	
18-9-24	5.70±0.77	
S-A-24		
Experiment III		
0-5-3	5.18±0.51	6.31±0.54
0-6-24-III	7.54±0.81	
3-5-3	7.12±0.63	6.48±0.47
3-6-24	5.84±0.64	
6-5-3	6.86±0.88	7.18±0.58
6-6-24	7.50±0.80	
9-5-3	8.66±1.04	7.84±0.67
9-6-24	7.02±0.80	

[a]Combined gamma globulin concentration data obtained after 5 and 6 days of repletion into one average value for each level of egg protein, 0%, 3%, 6%, and 9% egg protein.

Total RNA followed a pattern similar to that of spleen weight after repletion for both 5 and 6 days (Table 9). A linear relationship was observed between total splenic RNA and dietary egg protein from 0% to 9% egg after 6 days and from 0% to 6% egg for 5 days. The slopes of the regression equations were 0.232 and 0.178, respectively.

After 5 days similar mean values for RNA/g spleen were obtained for groups repleted with 3%, 6%, and 9% egg protein; these values were all significantly higher than that of the rats fed 0% egg protein (Table 9). Splenic RNA concentration of rats repleted 6 days differed significantly only between groups fed 9% egg and 0% egg diets and between rats fed 9% egg and 3% egg diets.

Linear relationships were found between total splenic DNA and dietary egg protein for groups repleted both 5 and 6 days. Total splenic DNA was increased about 0.50 mg and 0.42 mg for each 1% increment of dietary egg protein for the 5-day and 6-day repletion periods, respectively, up to 9% egg protein.

DNA concentrations of spleens were similar for all groups in both the 5- and 6-day repletion periods and appeared to be independent of the level of dietary egg protein (Table 9).

After 5 days of repletion RNA/DNA ratios were similar for all groups while rats repleted for 6 days with 0% egg protein diet had a significantly lower mean RNA/DNA ratio than the animals fed 9% egg (Table 9).

A comparison of changes occurring in the liver and spleen following repletion of protein-deficient rats showed that the spleen was generally more sensitive to dietary protein than the liver. Although raising the amount of dietary egg protein from 0% to 9% produced comparable gains in splenic and hepatic weight and total RNA for the 6-day repletion period, the spleen showed a greater gain in these parameters than the liver for the 5-day repletion period. The increase in total DNA with increase in dietary egg protein was much greater in the spleen than in the liver for both the 5- and 6-day repletion periods; therefore, greater increases were produced in hepatic RNA/DNA ratios than in splenic RNA/DNA ratios.

Mean agglutinin and hemolysin titers were similar for groups repleted for 5 days with 0%, 6%, and 9% egg protein; however, rats fed 3% egg had a significantly higher mean agglutinin titer than rats fed 6% and 9% egg and a significantly higher mean hemolysin titer than rats refed 0% egg protein. No significant differences in mean hemolysin or agglutinin titers were found after 6 days of repletion with 0% to 9% egg protein (Table 10).

Isolated antibody to sheep red blood cells

To estimate the portion of sheep red blood cell antibody that was recovered from the sera of rats in Experiments II and III, solutions of isolated antibody were titrated for hemolysin and agglutinin. Agglutinating activity was found in the anti-

body solutions, but no hemolytic activity could be demonstrated. Table 11 presents agglutinin titers of isolated antibody and the fraction of the total serum agglutinin titers that these titers represented. Agglutinating activity of antibody isolated from some animals could not be detected apparently due to either weak agglutinating capacity of the antibody or to dilution of the isolated antibody. Since the range of agglutinin titers for each group was so broad, median values were used as a basis for comparisons between groups of animals.

In Experiment III three hours after tracer injection, medians of agglutinin titers of antibody solutions were similar for groups repleted with 0% and 9% egg protein while slightly lower values were found with the 6% egg diet and somewhat higher values with 3% egg repletion. The fraction of total serum agglutinating activity present in the isolated antibody had median values ranging from 3% to 8% for groups refed 0% to 9% egg protein. However, the number of animals per group exhibiting demonstrable agglutinating activity ranged from 50% to 100% for these same repleted groups.

Twenty-four hours after tracer administration, the median agglutinin titers for isolated antibody solutions ranged from 0.69 for rats refed 6% egg to 1.22 for animals refed 0% egg with intermediate values for groups refed 3% and 9% egg. Titers of isolated antibody were demonstrated in 70 to 90% of the rats refed 0% to 9% egg protein 24 hours after tracer injection.

Table 11. Median log of agglutinin titers of isolated antibody and percentage of total serum agglutinin titer - Experiments II and III

Group	No. of rats	Tracer incorporation period hours	Rats per group exhibiting anti-body agglutinating activity %	Antibody solution agglutinin titer log	Fraction of total serum agglutinin titer %
Experiment II					
18-6-24	5	24	80	0.98(0-1.52)[a]	3(0-17)[a]
18-9-24	5	24	100	1.24(0.66-1.43)	8(5-9)
Experiment III					
0-5-3	10	3	70	0.86(0-2.00)	4(0-37)
3-5-3	10	"	100	1.37(0.78-1.68)	6(3-71)
6-5-3	10	"	50	0.35(0-1.62)	3(0-16)
9-5-3	10	"	70	0.95(0-1.58)	8(0-24)
0-6-24-III	10	24	80	1.22(0-1.96)	7(0-14)
3-6-24	10	"	90	1.01(0-1.32)	7(0-15)
6-6-24	10	"	70	0.69(0-1.69)	4(0-100)
9-6-24	10	"	80	0.89(0-1.34)	6(0-10)

[a]Range of values obtained for each group.

The fraction of total serum agglutinin titer present in the isolated antibody had median values ranging from 4% to 7% for groups refed 0% to 9% egg protein.

In Experiment II twenty-four hours after tracer injection, the median agglutinin titer of isolated antibody solutions was somewhat higher after 9 days of repletion with 18% egg protein than after 6 days. Recovery of total serum agglutinin activity was also somewhat greater after 9 days of repletion with 18% egg than after 6 days.

The inability to detect agglutinating activity in some of the isolated antibody solutions generally corresponded to low total agglutinin titers of the original serum. However, a high serum agglutinin titer did not always correspond to a high recovery of the titer in the isolated antibody solution.

Gamma Globulin

Since antibodies are primarily associated with gamma globulins, this serum fraction was examined to determine if variations in amount of dietary protein had any effect on its production. In addition, the immune response was compared with gamma globulin concentration. Serum concentration of gamma globulin was determined for groups fed 18% egg protein in Experiment II and for all groups in Experiment III (Table 10).

In Experiment III rats repleted for 5 days with 0% egg protein had significantly lower gamma globulin concentrations

than animals refed 3% or 9% egg, although values for rats refed 3%, 6%, and 9% egg were not statistically different from each other.

Gamma globulin concentration in serum was similar for all groups after 6 days of repletion.

Variation in gamma globulin concentration among groups in both the 5- and 6-day repletion periods did not follow the same patterns of change exhibited by antibody titers, hemolysins or agglutinins, by total spleen weight, or by total splenic RNA.

When gamma globulin concentrations for day 5 and day 6 were pooled for each experimental group, a regression equation showed a linear relationship between gamma globulin/ml serum and the amount of dietary egg protein. Serum gamma globulin concentration increased 0.18 mg/ml serum with each 1% increase in amount of dietary egg protein. Although the means of pooled day 5 and day 6 data for gamma globulin concentration at each protein level varied in a manner similar to that found for pooled splenic weight and splenic RNA means, no correlation was found between gamma globulin concentration and either splenic weight or splenic RNA.

Radioactivity Measurements

Serum

Since plasma concentrations of proteins reflect rates of synthesis and catabolism, turnover rates of serum gamma globulin

and antibody to sheep red blood cells were estimated to characterize more completely the immune response of rats repleted with 0% to 18% egg protein. Turnover of total serum proteins provided additional information on protein metabolism.

In Experiments II and III, ^{14}C-lysine was injected intravenously into rats during active synthesis of antibody in the primary immune response to sheep red blood cells. During a preliminary study rats were injected with 10 μc of ^{14}C-lysine and blood samples were taken 1, 2, 4, 8, 16, and 24 hours after tracer administration. Total serum and gamma globulin radioactivity appeared to be maximal at both 2 and 4 hours after tracer injection declined thereafter. Hence, serum which was obtained from rats 3 hours and 24 hours after tracer injection was used for calculations of incorporation rates and half-lives of total serum proteins, serum gamma globulin and antibody; the major activity at 3 hours was assumed to be due to synthesis and the difference between 3 and 24 hours due to catabolism.

Table 12 lists median values for radioactivity per volume of serum for whole serum, isolated gamma globulin, and isolated specific antibody to Srbc. Since the range of radioactivity values for each group was so broad, the median value was reported as it was more representative of the entire group of animals than the mean value. In Experiment II groups 18-6-24, 18-9-24, and S-A-24 had similar values for radioactivity per μl serum at 3 hours and 24 hours after tracer injection; these

Table 12. Radioactivity of ^{14}C-lysine/100 g body weight; median radioactivity per volume of serum based on tracer dose/100 g body weight for serum, gamma globulin, and isolated antibody; and per cent of serum radioactivity present in gamma globulin and isolated antibody - Experiments II and III

Experiment	Group	Dose: 100 g body weight μc/100 g	Serum radioactivity: cpm/μl serum	Serum radioactivity: (cpm/μl serum)/(dose/100 g)	Gamma globulin radioactivity (cpm/0.01 ml serum)/(dose/100 g)
II	0-6-24-II	2.60			
	3 hr		11.7 (8.8-16.5)*	4.48 (3.39-6.36)**	
	24 hr		9.3 (7.1-11.0)	3.56 (2.74-4.25)	
	18-6-24	2.39			
	3 hr		10.6 (8.2-22.7)	4.45 (3.43-9.50)	
	24 hr		6.4 (5.0-12.9)	2.68 (2.09-5.40)	3.2 (1.8-6.6)
	18-9-24	2.27			
	3 hr		9.9 (7.8-12.8)	4.34 (3.43-5.63)	
	24 hr		5.7 (5.0-7.1)	2.52 (2.20-3.14)	2.3 (1.5-3.2)
	S-A-24	1.86			
	3 hr		9.2 (7.8-23.1)	4.94 (4.17-12.41)	
	24 hr		5.0 (4.1-10.8)	2.71 (2.19-5.78)	
III	0-5-3	2.67	11.8 (8.8-49.7)	4.40 (3.29-18.6)	3.7 (2.8-16.9)
	0-6-24-III	2.74	10.1 (9.1-12.6)	3.68 (3.31-4.62)	4.4 (2.9-8.9)
	3-5-3	2.51	10.4 (5.4-13.1)	4.16 (2.16-5.24)	3.3 (1.7-5.7)
	3-6-24	2.50	8.1 (4.6-12.5)	3.24 (1.75-5.03)	3.1 (2.4-4.3)
	6-5-3	2.37	12.0 (7.6-17.2)	5.05 (3.19-7.28)	3.9 (2.2-8.6)
	6-6-24	2.46	9.0 (3.4-20.1)	3.64 (1.39-8.14)	3.7 (1.9-8.2)
	9-5-3	2.38	11.9 (4.9-17.9)	4.98 (2.06-7.52)	4.2 (2.1-5.6)
	9-6-24	2.40	8.8 (6.1-15.5)	3.68 (2.53-6.45)	2.7 (1.3-7.2)

*Range of values for each group.

**Statistical analyses: Medians of groups with the same numbered superscript in a horizontal row are not statistically different ($P < 0.05$). Comparisons made among groups in Experiment II, among 5-day repleted groups in Experiment III and among 6- and 9-day repleted groups in (footnote continued on following page)

Table 12 (Continued)

Experiment	Group	Isolated antibody radioactivity (cpm/ml serum) (dose/100 g)	Per cent serum radioactivity in gamma globulin %	Per cent serum radioactivity in isolated antibody %
II	18-6-24 24 hr	7.5 (6.2-11.2)	11 (8-15)	0.33 (0.11-0.39)
	18-9-24 24 hr	4.1 (2.3-8.4)	9 (6-13)	0.13 (0.10-0.32)
III	0-5-3	11.9 (8.6-38.8)	9 (6-9)	0.26 (0.17-0.63)
	0-6-24-III	7.7 (4.3-16.2)	12 (8-18)	0.21 (0.11-0.44)
	3-5-3	10.0 (5.6-19.5)	9 (6-15)	0.28 (0.13-0.52)
	3-6-24	6.9 (2.9-11.3)	10 (6-18)	0.18 (0.11-0.61)
	6-5-3	8.0 (4.6-22.4)	8 (4-15)	0.17 (0.11-0.37)
	6-6-24	8.9 (3.2-26.0)	9 (6-18)	0.16 (0.09-1.90)
	9-5-3	8.7 (1.6-50.6)	8 (4-11)	0.20 (0.03-0.97)
	9-6-24	7.1 (3.6-24.6)	8 (3-18)	0.20 (0.10-0.63)

(footnote continued from previous page) Experiments II and III.

Serum radioactivity-(cpm/µl serum)/(dose/100 g body weight):

3-5-3[1], 0-5-3[1,2], 9-5-3[2], 6-5-3[2]

24-hour values: {18-9-24[1],18-6-24[1],S-A-24[1],3-6-24[1,2],0-6-24-II[2],6-6-24[2], 0-6-24-III[2], 9-6-24[2]

Gamma globulin radioactivity:

18-9-24[1],9-6-24[1],3-6-24[1],18-6-24[1,2],6-6-24[1,2],0-6-24-III[2]

Isolated antibody radioactivity:

6-5-3[1], 9-5-3[1,2], 3-5-3[1,2], 0-5-3[2]

values were significantly lower than that found for group 0-6-24-II for the 24 hour period only. Although all groups received similar doses of ^{14}C-lysine, 10 µc, rats repleted with 18% egg protein and those fed the stock ration were larger than animals refed 0% egg; hence, the dose per 100 g body weight was not the same for all groups (Table 12). Since plasma volume is proportional to body weight, the tracer dose was diluted to a greater extent in groups refed 18% egg protein and the stock diet than in the group fed 0% egg protein. However, when direct comparisons were made between groups using (cpm/µl serum) per (dose/100 g body weight) the same differences were found among the groups as when cpm/µl serum was used as the basis for comparison.

Serum radioactivity/µl serum based on tracer dose/100 g body weight was reduced about 40% more between 3 and 24 hours after tracer injection in rats fed egg protein and stock ration than in animals repleted with 0% egg. Since serum radioactivity 3 and 24 hours after tracer administration is primarily associated with serum proteins and not with free amino acids (Schultze and Heremans, 1966), total serum proteins were turning over more rapidly in those animals fed 18% egg protein or stock diet than in those fed 0% egg.

In Experiment III median values of radioactivity/µl serum were similar for all groups 3 hours after tracer injection and comparable reductions in radioactivity were observed for all

groups after an additional 21 hours. However, 3 hours after tracer injection median values of (cpm/µl serum) per (dose/100 g body weight) were significantly lower in rats refed 3% egg protein than in animals given 6% or 9% egg but not 0% egg. Twenty-four hours after tracer administration when median values for serum radioactivity based on dose/100 g body weight were estimated, they were similar for all groups. Hence, reduction in serum radioactivity from 3 hours to 24 hours after tracer injection was greater in groups refed 6% egg, 28% decrease, and 9% egg, 26% decrease, than in groups refed 0% egg, 16% decrease and 3% egg, 20% decrease.

Total serum radioactivity was estimated to determine if the differences among groups based on radioactivity/µl serum values were due to variation in blood volumes among groups (Table 13). Unpublished work[1] in this laboratory, using Evans blue dye to determine blood volume, has shown that adult male rats treated similarly to animals in Experiments II and III had mean plasma volumes of 4.0 ml/100 g body weight when repleted with 0% egg protein, 3.8 ml/100 g after repletion with 9% egg protein and 3.6 ml/100 g when fed stock ration continaully. These values were used to estimate plasma volumes for similar groups in this study. Using these values as a basis the following approximations of plasma volume were made for the other

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[1]Alberta Paysen determined blood volumes in an FN 415 project.

Table 13. Calculated plasma volume and median total radioactivity values for serum, gamma globulin, isolated antibody, and excreta (urine + feces) - Experiments II and III

Experiment	Group	Plasma volume ml
II	0-6-24-II	15.4 (14.6-16.0)*
	3 hr	
	24 hr	
	18-6-24	15.5 (14.5-16.4)
	3 hr	
	24 hr	
	18-9-24	16.3 (15.6-16.8)
	3 hr	
	24 hr	
	S-A-24	20.8 (8.9-24.8)
	3 hr	
	24 hr	
III	0-5-3	15.0 (14.1-15.8)
	0-6-24-III	14.6 (13.2-16.4)
	3-5-3	15.5 (14.6-16.2)
	3-6-24	15.5 (13.4-16.3)
	6-5-3	16.0 (14.3-17.2)
	6-6-24	15.6 (15.0-16.3)
	9-5-3	15.9 (15.0-16.5)
	9-6-24	15.8 (13.5-16.9)

*Range of values from which the median was derived.

Table 13 (Continued)

Total radioactivity in serum cpm/1000	Total radioactivity in gamma globulin cpm/1000	Total radioactivity in isolated antibody cpm/100
178.6 (138.1-262.2)**		
138.6 (109.0-175.2)		
165.0 (133.1-346-4)		
103.8 (81.1-197.1)	12.3 (6.7-24.1)	2.96 (2.69-3.88)
159.6 (121.9-211.8)		
91.8 (79.9-118.2)	8.5 (5.4-11.7)	1.52 (0.82-2.96)
195.1 (166.3-530.4)		
101.1 (86.7-246.9)		
180.0 (124.0-761.8)	15.2 (10.5-69.1)	4.89 (3.45-15.9)
144.0 (135.7-199.6)	17.8 (11.8-30.2)	3.30 (1.80-6.01)
156.6 (83.9-212.8)	13.0 (6.9-22.5)	3.81 (2.14-7.76)
126.1 (61.8-197.2)	12.3 (9.4-16.8)	2.66 (1.17-3.78)
189.8 (46.6-322.5)	15.2 (9.2-32.1)	3.14 (1.70-8.28)
138.7 (119.7-281.3)	14.0 (7.2-32.7)	3.41 (1.23-8.76)
183.6 (79.4-269.5)	15.8 (8.4-22.1)	3.40 (0.60-18.6)
139.6 (87.6-247.4)	10.7 (4.8-25.1)	2.87 (1.43-8.54)

**Statistical analyses; Medians of groups with the same numbered superscript in a horizontal row are not statistically different ($P < 0.05$). Comparisons made among all groups in Experiment II, among 5-day repleted groups in Experiment III and among 6- and 9-day repleted groups in Experiments II and III.

Total serum radioactivity: 3-5-3^{1}, 9-5-3^{1,2}, 0-5-3^{1,2}, 6-5-3^{2}

24 hour values: {18-9-24^{1}, S-A-24^{1}, 18-6-24^{1}, 3-6-24^{1,2}, 0-6-24-II^{2}, 6-6-24^{2}, 9-6-24^{2}, 0-6-24-III^{2}

Total gamma globulin radioactivity: 18-9-24^{1}, 9-6-24^{1,2}, 18-6-24^{1,2,3}, 3-6-24^{2}, 6-6-24^{1,2,3}, 0-6-24-III^{3}

(footnote continued on following page)

Table 13 (Continued)

Experiment	Group	Adjusted total[a] radioactivity in isolated antibody cpm/100	Total radioactivity in excreta cpm/1000
II	0-6-24-II		
	24 hr		125.3 (63.7-210.5)
	18-6-24		
	24 hr	98.6 (75.4-129)	133.0 (77.5-264.1)
	18-9-24		
	24 hr	19.1 (10.3-37.0)	130.4 (83.4-142.5)
	S-A-24		
	24 hr		153.6 (82.5-455.4)
III	0-5-3	122.0 (86.3-397)	48.6 (26.5-97.9)
	0-6-24-III	47.1 (25.7-86.8)	113.6 (81.7-149.8)
	3-5-3	63.6 (35.8-129)	43.6 (17.6-115.9)
	3-6-24	38.0 (16.7-54.1)	128.6 (55.4-205.3)
	6-5-3	104.5 (56.6-276)	68.0 (40.7-133.1)
	6-6-24	84.9 (30.8-218)	107.7 (62.8-216.9)
	9-5-3	42.6 (7.5-233)	48.4 (22.2-105.3)
	9-6-24	48.0 (23.9-142.2)	127.3 (82.9-159.1)

(footnote continued from previous page)

Total antibody radioactivity: 6-5-3[1], 9-5-3[1,2], 3-5-3[1,2], 0-5-3[2]

Adjusted total antibody radioactivity: 9-5-3[1], 3-5-3[1], 6-5-3[2], 0-5-3[2]

24 hour values: 18-9-24[1], 3-6-24[2], 0-6-24-III[2], 9-6-24[2,3], 6-6-24[3,4], 18-6-24[4]

Total excreta radioactivity: 6-6-24[1], 0-6-24-III[1], 0-6-24-II[1], 9-6-24[1], 3-6-24[1,2], 18-9-24[1,2], 18-6-24[1,2], S-A-24[2]

[a]Adjusted according to the percent of antibody recovered in the isolation procedure by multiplying each individual antibody radioactivity value by the median percent recovery for each respective group.

groups in Experiments II and III: 1) repletion with 3% egg - 3.9 ml/100 g body weight, 2) 6% egg repletion - 3.8 ml/100 g body weight, and 3) repletion with 18% egg - 3.7 ml/100 g body weight.

In Experiment II mean calculated plasma volumes were similar for groups repleted with 0% egg protein and 18% egg (Table 13). Stock-fed animals had the largest mean plasma volume for all groups. Three hours after tracer injection total serum radioactivity exhibited a pattern similar to that observed for (serum radioactivity/µl serum) per (dose/100 g body weight) (Tables 12 and 13). Twenty-one hours later, the same relative reductions in radioactivity occurred for total serum values as for (radioactivity/µl serum) per (dose/100 g body weight).

In Experiment III plasma volumes were similar for all groups (Table 13). Median values calculated for total serum radioactivity and for the decrease in radioactivity from 3 to 24 hours after tracer injection followed the same patterns as those based on concentration per (dose/100 g body weight) (Tables 12 and 13).

Gamma globulin

In Experiment III three hours after tracer injection, median values for gamma globulin expressed as (radioactivity/µl serum) per (dose/100 g body weight) were statistically similar for all groups whether repleted with egg protein or

not (Table 12). Reductions in gamma globulin radioactivity were observed for groups repleted with 3%, 6%, and 9% egg protein during the next 20 hours, while animals refed 0% egg exhibited a 20% increase in radioactivity (Table 12). The greatest reduction, 35%, in gamma globulin radioactivity occurred in animals fed 9% egg while radioactivity in gamma globulin of rats fed 3% egg and 6% egg decreased 8% and 6%, respectively. Values for total globulin radioactivity at both 3 hours and 24 hours after tracer injection paralleled those observed for (radioactivity values/µl serum) per (dose/100 g body weight) (Tables 13 and 14).

In Experiment II, 24 hours after tracer administration, gamma globulin expressed as (cpm/µl serum) per (dose/100 g body weight) for animals repleted 6 days with 18% egg was similar to that of animals fed 0% to 9% egg protein for 6 days in Experiment III, i.e., 3.2 versus 2.7 to 4.4. Repletion with 18% egg for 9 days produced a lower median value for gamma globulin activity, 2.3 (cpm/µl serum) per (dose/100 g body weight), which was significantly lower than the median value of the group refed 0% egg in Experiment III. Median values for total gamma globulin radioactivity with repletion changed in the same way (Table 13).

The fraction of (radioactivity/µl serum) per (dose/100 g body weight) due to gamma globulin was similar for all groups 3 hours after tracer injection, 8% to 9% in Experiment III.

Table 14. Median values for specific activity of gamma globulin and isolated antibody - Experiments II and III

Experiment	Group	Specific activity of gamma globulin cpm/0.1 mg	Specific activity of isolated antibody cpm/agglutinin titer
II	18-6-24 24 hr	12 (6-25)*,**	1.16 (0-4.50)
	18-9-24 24 hr	10 (7-11)	0.46 (0.37-4.76)
III	0-5-3	20 (11-129)	2.32 (0-14.8)
	0-6-24-III	16 (10-26)	1.60 (0-5.23)
	3-5-3	12 (8-21)	1.62 (0.61-4.07)
	3-6-24	15 (8-23)	1.36 (0-3.20)
	6-5-3	18 (6-26)	0.25 (0-7.19)
	6-6-24	11 (9-25)	0.66 (0-7.68)
	9-5-3	12 (6-23)	1.36 (0-3.72)
	9-6-24	11 (6-20)	1.80 (0-9.84)

*Range of values from which the median was derived.

**Statistical analyses: Medians of groups with the same numbered superscript in a horizontal row are not statistically different ($P < 0.05$). Comparisons made among 5-day repleted groups in Experiment III and among 6- and 9-day repleted groups in Experiments II and III.

Gamma globulin specific activity: $9\text{-}5\text{-}3^{1}$, $3\text{-}5\text{-}3^{1}$, $6\text{-}5\text{-}3^{1,2}$, $0\text{-}5\text{-}3^{2}$ $18\text{-}9\text{-}24^{1}$, $9\text{-}6\text{-}24^{1,2}$, $6\text{-}6\text{-}24^{1,2}$, $18\text{-}6\text{-}24^{1,2}$, $3\text{-}6\text{-}24^{2}$, $0\text{-}6\text{-}24\text{-III}^{2}$

Isolated antibody specific activity: No significant differences.

Twenty-four hours after administration of tracer, the percentages of serum radioactivity due to gamma globulin for groups fed 0% to 9% egg in Experiment III were similar to each other and to those fed 18% egg in Experiment II, i.e., 8% to 12%.

Three hours after tracer injection in Experiment III, the median specific activity of serum gamma globulin for animals refed 0% egg protein, 20 cpm/0.1 mg gamma globulin, was significantly higher than that of groups refed 3% and 9% egg, 12 cpm/0.1 mg for both groups, but not different from that of animals refed 6% egg, 18 cpm/0.1 mg (Table 14). Twenty-four hours after tracer administration, the only significant difference in gamma globulin specific activity occurred between groups refed 0% and 9% egg protein, 16 cpm/0.1 mg and 11 cpm/0.1 mg, respectively.

In Experiment II, 24 hours after tracer administration, specific activities of gamma globulin for groups repleted with 18% egg protein for 6 and 9 days were similar, 12 and 10 cpm/0.1 mg, respectively. Only 18% egg for 9 days produced a value for gamma globulin specific activity which was significantly lower than those of groups refed 0% and 3% egg in Experiment III.

Isolated antibody

Three hours after tracer injection in Experiment III (isolated antibody radioactivity/ml serum) per (dose/100 g body weight) was significantly higher for rats refed 0% egg

protein than for rats given 6% egg. Twenty-one hours later values for isolated (antibody radioactivity/ml serum) per (dose/100 g body weight) were similar for all groups in Experiment III and for 2 groups fed 18% egg in Experiment II (Table 12).

The fraction of radioactivity/ml serum that could be attributed to isolated antibody 3 hours after tracer injection was similar for groups repleted with 0%, 3%, 6%, and 9% egg, 0.26%, 0.28%, 0.17%, and 0.20%, respectively. Twenty-one hours later the median percentage of serum radioactivity accounted for by isolated antibody decreased somewhat or remained the same; 0.21%, 0.18%, 0.16%, and 0.20% for 0%, 3%, 6%, and 9% egg, respectively. The percentage of (radioactivity/ml serum) per (dose/100 g body weight) due to isolated antibody in rats repleted with 18% egg for 6 days, 0.33%, and for 9 days, 0.13%, were not significantly different from the values observed for repleted groups in Experiment III after similar 24 hour incorporation periods (Table 12).

Total antibody radioactivity present in the serum and total antibody radioactivity, corrected for low recovery of antibody during the antibody isolation procedure, are given in Table 13. In Experiment III, 3 hours after tracer administration, total radioactivity present in the agglutinin fraction was significantly different only between rats refed 0% and 6% egg protein.

Twenty-four hours after injection of tracer, total agglutinin radioactivity was similar for all groups in Experiment III and for groups refed 18% egg in Experiment II (Table 13).

When total agglutinin radioactivity was corrected for low recovery of antibody during the isolation procedure, a somewhat different pattern of response was obtained than that observed for unadjusted total agglutinin radioactivity. Three hours after tracer injection, adjusted total agglutinin radioactivity was significantly greater in groups fed 0% and 6% egg protein than in groups given 3% and 9% egg protein. However, adjusted total agglutinin radioactivity 24 hours after tracer administration was significantly higher in rats refed 6% egg than in animals given 0% and 3% egg protein.

In Experiment II, 24 hours after tracer injection, adjusted total agglutinin radioactivity of animals refed 18% egg for 9 days was significantly lower than the median values of all groups in Experiment III for the same incorporation period. However, 18% egg for 6 days produced a median value which was significantly greater than all groups except the rats refed 6% egg in Experiment III for the 24-hour incorporation period.

The specific activity of the isolated antibody fraction was estimated from the agglutinin titer and the radioactivity of the isolated antibody solution (Table 14). Since the range of values for specific activity of isolated antibody were so broad for each group in Experiment II and for groups refed 18%

egg in Experiment II, no significant differences could be shown among the groups either 3 hours or 24 hours after tracer injection.

Excreta radioactivity

In Experiment III, the medians of total radioactivity excreted in the urine and feces combined were similar for all groups 3 hours after tracer injection 43.6 to 68.0 cpm/1000, and also 24 hours after tracer administration, 107.7 to 128.6 cpm/1000 (Table 13). In Experiment II, 24 hours after tracer injection, medians of total radioactivity excreted were similar for groups refed 0% and 18% egg protein. However, rats fed stock ration throughout the experiment excreted significantly more radioactivity than the group refed 0% egg and also significantly more radioactivity than groups refed 0%, 6%, and 9% egg in Experiment III.

Distribution of dose radioactivity

Table 15 lists the percentages of the radioactivity of the initial dose of L-lysine-^{14}C which were present in the serum, serum gamma globulin fraction, antibody fraction, and in the excreta of groups in Experiments II and III, either 3 or 24 hours after injection of the tracer.

In Experiment II, 5 to 6% of the injected radioactivity was present in the serum of all groups 3 hours after tracer administration. Twenty-four hours after tracer injection, serum radioactivity had decreased to about 3% of the initial

Table 15. Median values for percentage of initial radioactivity of L-lysine-^{14}C dose present in serum, gamma globulin, isolated antibody and in excreta (urine + feces) - Experiments II and III

Experiment	Group	Serum radioactivity %	Gamma globulin radioactivity %	Antibody radioactivity %
II	0-6-24-II			
	3 hr	5.8 (4.1-7.9)*		
	24 hr	4.5 (3.3-5.5)		
	18-6-24			
	3 hr	5.2 (4.0-11.2)		
	24 hr	3.3 (2.4-6.4)	0.38 (0.20-0.78)	0.010 (0.007-0.012)
	18-9-24			
	3 hr	5.0 (3.9-6.4)		
	24 hr	2.9 (2.5-3.6)	0.27 (0.17-0.38)	0.004 (0.003-0.010)
	S-A-24			
	3 hr	5.9 (5.4-15.9)		
	24 hr	3.2 (2.8-7.4)		
III	0-5-3	5.6 (3.7-24.6)	0.45 (0.30-2.20)	0.015 (0.010-0.051)
	0-6-24-III	4.4 (4.1-6.0)	0.54 (0.36-0.91)	0.010 (0.005-0.018)
	3-5-3	4.8 (2.5-6.4)	0.39 (0.21-0.73)	0.012 (0.006-0.025)
	3-6-24	3.8 (2.0-6.2)	0.37 (0.29-0.54)	0.008 (0.004-0.012)
	6-5-3	5.9 (3.9-8.7)	0.47 (0.26-0.96)	0.010 (0.005-0.025)
	6-6-24	4.3 (1.5-9.7)	0.42 (0.22-0.98)	0.010 (0.004-0.028)
	9-5-3	5.6 (2.6-8.1)	0.48 (0.25-0.66)	0.010 (0.002-0.056)
	9-6-24	4.2 (2.6-8.0)	0.32 (0.15-0.75)	0.018 (0.004-0.026)

*Range of values for each group from which the median was derived.

Table 15 (Continued)

Experiment	Group	Antibody radioactivity adjusted for % recovery %	Excreta radioactivity %
II	0-6-24-II		
	24 hr		3.7 (2.1-5.1)
	18-6-24		
	24 hr	0.07 (0-0.27)	4.0 (2.5-7.9)
	18-9-24		
	24 hr	0.08 (0.04-0.17)	3.9 (2.7-4.6)
	S-A-24		
	24 hr		5.0 (2.7-13.7)
III	0-5-3	0.16 (0-1.98)	1.5 (0.5-3.2)
	0-6-24-III	0.10 (0-0.39)	3.5 (2.4-4.5)
	3-5-3	0.19 (0.02-0.45)	1.4 (0.6-3.5)
	3-6-24	0.11 (0-0.19)	4.0 (1.7-6.2)
	6-5-3	0.03 (0-0.33)	2.2 (1.3-4.0)
	6-6-24	0.07 (0-0.79)	3.0 (1.9-6.5)
	9-5-3	0.08 (0-0.17)	1.5 (0.7-3.2)
	9-6-24	0.11 (0-0.64)	3.8 (2.7-5.2)

dose in animals repleted with 18% egg or fed the stock diet while animals refed 0% egg retained about 4.5% of the initial dose radioactivity in their sera.

In Experiment III after 3 hours of tracer incorporation, serum radioactivity represented approximately 5 to 6% of the injected radioactivity for all groups. Twenty-one hours later approximately 4% of initial dose radioactivity was present in the sera of all groups.

Twenty-four hours following tracer injection in Experiment II, the fraction of the initial dose of radioactivity present in gamma globulin was similar for groups repleted for 6 and 9 days with 18% egg protein, 0.27% and 0.38%. In Experiment III the percentage of the initial dose present in gamma globulin 3 hours after tracer administration was similar for all groups, 0.39% to 0.48%. After 24 hours of tracer incorporation, the fraction of radioactivity in gamma globulin was essentially the same for all groups and did not differ greatly from the 3-hour values.

The percentage of radioactivity present in the antibody fraction of the serum was corrected for the low recovery of antibody during the antibody isolation procedure and the adjusted values as well as the actual measured values for each group are given in Table 16. In Experiment III the percentage of radioactivity present as antibody 3 hours after tracer injection was similar for groups refed 0% egg and 3% egg, 0.16%

Table 16. Calculated half-lives ($T_{1/2}$) for total serum proteins, gamma globulin and antibody of rats repleted with 0%, 3%, 6%, 9% or 18% egg protein - Experiments II and III

Group	Egg protein during repletion %	Total serum proteins half-life days		Gamma globulin half-life days		Antibody half-life days		
		A[a]	B[b]	A[a]	B[b]	A[a]	B[b]	C[c]
0-6-24-II	0	2.9	3.0					
18-6-24	18	1.6	1.5					
18-9-24	18	1.4	1.4					
S-A-24	stock ration	1.3	1.3					
0-5-3 0-6-24-III	0	3.0	3.7	-	-	1.9	1.7	1.0
3-5-3 3-6-24	3	3.1	2.8	11.8	7.8	2.0	2.0	1.5
6-5-3 6-6-24	6	2.3	2.2	7.5	8.5	-	-	3.2
9-5-3 9-6-24	9	2.5	2.3	1.9	1.7	3.9	3.3	-

[a]Calculated from total radioactivity values.

[b]Calculated from (radioactivity/ml serum)/(dose/100 g body weight).

[c]Calculated from total radioactivity values adjusted for % recovery of isolated antibody during the isolation procedure.

and 0.19%, respectively, while lower values were observed for rats given 6% egg and 9% egg, 0.03% and 0.08%, respectively. Twenty-four hours after tracer administration 0.07% to 0.11% of dose radioactivity was present in antibody of groups refed 0% to 9% egg protein.

In Experiment II, 24 hours after tracer injection, the percentage of dose radioactivity present in antibody was similar for the groups repleted with 18% egg for 6 and 9 days, 0.010% and 0.005%, respectively.

The fraction of initial injected radioactivity excreted in urine and feces during the 24-hour period following tracer injection in Experiment II ranged from 3.7% for rats fed 0% egg to 5.0% for animals refed stock ration. In Experiment III the percentage of radioactivity excreted 3 hours after tracer administration was 1.5%, 1.4%, and 1.5% for groups refed 0% egg, 3% egg, and 9% egg, respectively, while a slightly higher value, 2.2%, was observed with 6% egg repletion. During the 24 hour period following tracer injection the percentage of radioactivity in excreta increased to a similar level for all groups, 3.0% to 4.0% (Table 15). Respiratory $^{14}CO_2$ losses were not measured.

Turnover of Serum Proteins

"Half-lives" of total serum proteins, serum gamma globulin and isolated antibody were estimated for animals in Experiments

II and III from radioactivity data collected 3 and 24 hours after tracer injection (Table 16). "Half-lives" and "half-life" throughout the text refer to approximate values derived from the decrease in radioactivity between 3 and 24 hours after tracer injection.

Synthesis and catabolism of plasma proteins appear to be first-order reactions, i.e., the amount of substrate converted, dS, during a period of time, dt, is directly proportional to the concentration of substrate present (Schultze and Heremans, 1966). This relationship may be written as $dS/dt = kS$ and integration of this equation produces the expression $S_t = S_o e^{-kt}$ which relates the amount of untransformed material left at time t to the initial amount of material. Hence, plotting the function of S versus t on semi-logarithmic paper produces a straight line whose slope measures the fractional turnover rate k. "Half-lives" were estimated using the expression "half-life" = $\frac{0.693}{k}$ which was derived from $S_t = S_o e^{-kt}$ when $S_t = 1/2\ S_o$.

The fractional rate of turnover was determined in this study by relating the change in radioactivity which occurred between the 3-hour and 24-hour incorporation periods to the initial radioactivity present 3 hours after tracer injection. "Half-lives" were calculated from the fractional rates of turnover in Experiment II for each individual rat while in Experiment III these parameters were based on the median value of one group in which blood was taken 3 hours after tracer injection

and of a second group in which blood was taken after 24 hours.

In Experiment II rats repleted with 18% egg or fed the stock ration had similar short "half-lives" for total serum proteins, 1.3 days to 1.6 days, while a longer mean "half-life" was found for animals refed with 0% egg, 2.9 days. Similar results were obtained using either radioactivity/ml serum based on the tracer dose per body weight or using total radioactivity data.

When total serum radioactivity values were used to calculate the "half-life" of total serum proteins in animals repleted with 0% egg in Experiment III, the value was 3 days; a slightly longer half-life, 3.7 days, was obtained from estimates based on radioactivity/ml serum adjusted for dose per 100 g body weight. Repletion with 3% egg indicated a "half-life" for serum proteins of approximately 3 days, calculated from either radioactivity/ml serum per dose or from total serum data. Animals repleted with 6% egg and 9% egg had estimated "half-lives" for total serum proteins of 2.3 days and 2.5 days, respectively, calculated from total serum radioactivity data. Similar results were obtained using radioactivity data/ml serum.

The calculated "half-life" of gamma globulin in Experiment III for animals repleted with 3% egg was approximately 12 days using total radioactivity data and about 8 days using radioactivity/ml serum based on tracer dosage. Calculated on either basis, the estimated "half-lives" of gamma globulin were

approximately 8 days and 2 days for groups refed 6% and 9% egg, respectively. Since gamma globulin radioactivity for 0% egg-repleted rats increased between 3 and 24 hours after tracer injection, gamma globulin "half-life" could not be calculated.

"Half-lives" of isolated antibody for groups refed 0% egg and 3% egg were approximately 2 days, calculated from data based either on total radioactivity or on radioactivity/ml serum adjusted for tracer dosage per 100 g body weight. The calculated "half-life" of isolated antibody in animals refed 9% egg was about 4 days based on total radioactivity values and slightly greater than 3 days using values for radioactivity/ml serum. Isolated antibody radioactivity in rats refed 6% egg was still increasing 24 hours after tracer injection, hence, antibody "half-life" could not be estimated.

Total antibody radioactivity values were adjusted for the per cent recovery of isolated antibody during the isolation procedure. When these adjusted values were used to calculate half-lives of isolated antibody, somewhat different estimations were obtained than those reported above. Half-lives of isolated antibody were approximately 1 day for groups refed 0% egg, 1.5 days for 3% egg repletion, approximately 3 days for 6% repletion and unknown for 9% egg repletion since antibody radioactivity 24 hours after tracer injection was greater than the 3 hour value.

DISCUSSION

All cells of the body synthesize proteins, both for the purpose of enzymatic reactions and also as a construction material. The primary site of protein synthesis in the cell is the ribosomes; however, incorporation of amino acids into protein has been demonstrated in mitochondria (Truman and Korner, 1962) and cell nuclei (Logan et al., 1959).

Body proteins are in a dynamic state or a constant state of change; proteins continuously are broken down and resynthesized. External factors such as the dietary supply of amino acids, fat, and carbohydrate have been shown to influence the protein content of body tissues (Munro, 1964). In addition, hormones and their interaction with the diet have modified protein metabolism (Leathem, 1964). Hence, the internal processes of the cell such as protein metabolism rely, in part, on the extracellular environment.

Protein deficiency has different effects in various body tissues; for example, liver and muscle have been shown to be more susceptible to alterations in dietary protein than tissues such as kidney and brain (Allison et al., 1962). In addition, repletion of different body tissues does not occur at the same rate (Neuberger and Richards, 1964). Hence, a commonly used determination of nutritive value of proteins such as change in body weight may not reveal imbalances in the distribution of body nitrogen in various tissues. Measurements of hepatic and

splenic nucleic acids, circulating antibodies, and half-lives or turnover rates of plasma proteins may provide a more discriminating assessment of the adequacy of dietary proteins and protein nutriture with respect to the needs of individual tissues. These parameters were chosen for this study because they might elucidate aspects of the immune response or antibody-producing process which are affected by variations in dietary protein.

The Biological Value of whole egg proteins is 100; i.e., at low intakes virtually 100% of the absorbed amino acids are utilized for tissue growth or replenishment (Allison, 1964). However, the Biological Value or the nutritive value of a protein may vary with the physiological state of the individual. During antibody production, for example, amino acid and/or protein requirements may be altered from those necessary for growth or maintenance. In this study, different levels of egg protein were evaluated in terms of rat requirements for tissue replenishment and antibody production.

Weight Gains and Nitrogen Efficiency Ratio

Repletion of protein-calorie depleted rats with 0% to 18% egg protein increased body weight as well as weights of liver and spleen. Weight gains increased as the dietary protein was raised from 0% to 6%. Raising dietary protein to 9% or 18% egg

produced no further significant increments in body weight gain.

Nitrogen efficiency ratios decreased significantly as the amount of dietary egg protein was raised. Egg protein is nearly completely retained in the adult animal receiving less nitrogen than is needed to bring him into nitrogen equilibrium; however, when larger amounts than this are given, the utilization of the nitrogen falls off progressively. The efficiency with which egg protein was utilized was maximal at 3% and decreased progressively as the protein was increased to 18%. Actual weight gain during repletion was greater with 6% egg than with 3% but further increases in dietary protein did not increase weight gain any further.

The actual amino acid intake of groups repleted with egg protein are compared in Table 17 with the minimum requirements of an adult depleted rat for maximal rate of repletion when the diet contained 10% protein (Steffee et al., 1950). With respect to the rat's requirements, the egg diets were most deficient in isoleucine, followed closely by lysine. Egg protein at the 3% level supplied roughly 1/3 of the amino acids required while 6% and 9% egg provided about 1/2 and about 3/4 respectively. Repletion with 18% egg provided all essential amino acids in excess of the requirement for maintenance and repletion of the rat.

Table 17. Percentage of requirements of essential amino acids for repletion furnished by different egg diets[a] - Experiments II and III

	Lysine %	Histidine %	Phenylalanine and tyrosine %	Tryptophan %
Requirement (mg/day)[b]	158.4	57.6	122.4	38.4
Experiment II				
18-6-24	127.1	131.5	259.1	135.0
18-9-24	128.6	132.7	262.2	136.8
Experiment III				
3-5-3 3-6-24	25.5	26.4	52.0	27.1
6-5-3 6-6-24	48.2	49.6	98.3	51.4
9-5-3 9-6-24	70.4	72.6	144.0	74.8

[a]Amino acid composition of whole egg was taken from Orr and Watt, 1957.

[b]Estimated for 480 g protein-depleted rat from Steffee et al., 1950.

Table 17 (Continued)

	Methionine and cystine %	Threonine %	Leucine %	Isoleucine %	Valine %
Requirement (mg/day)	105.6	117.6	199.2	165.5	139.2
Experiment II					
18-6-24	163.5	133.1	139.2	126.3[c]	167.9
18-9-24	165.5	134.9	141.5	127.6	170.0
Experiment III					
3-5-3 3-6-24	32.4	26.7	27.9	25.3	33.7
6-5-3 6-6-24	61.9	50.6	52.8	48.0	63.6
9-5-3 9-6-24	90.4	73.8	77.2	70.0	93.0

[c]Lowest percentage is underlined.

Liver and Hepatic Nucleic Acids

Protein-calorie depletion for 3 weeks produced greater losses in liver weight than did ad libitum protein depletion (Experiment I). However, protein depletion appeared to effect greater weight losses for the adrenal glands and kidneys while both depletion regimens produced comparable weight reductions for spleens and testes. When organ weights were based on 100 g body weight, reductions in weights of the kidneys and adrenal glands were proportional to loss in total body weight with both protein and protein-calorie depletion. Losses in mean splenic weight per 100 g body weight were proportionately greater and losses in mean testicular weight per 100 g body weight proportionately less than total body weight reductions irrespective of the depletion regimen. Hepatic weight losses were greater than losses in body weight during protein-calorie depletion and less during protein depletion. Hence, the testes were maintained during both depletion regimens in contrast to the other organs. Immunization had no apparent effect on organ weights during depletion. Therefore, protein-calorie depletion for 3 weeks was considered a suitable procedure for depleting in succeeding experiments.

The larger liver weights of protein-depleted rats compared with protein-calorie-depleted animals was probably due to the deposition of hepatic fat during the protein-depletion period. In 1961, Sidransky and Clark reported that increasing the energy

value of the dietary intake of malnourished rats caused an increased liver weight due to deposition of fat in the liver.

Repletion of protein-calorie depleted rats with 0% egg protein or 3% to 18% egg protein produced significant increases in liver weight which were progressive to the 6% egg level. Feeding 9% egg for 6 days produced only a slight additional increment in liver weight while 9% egg for 5 days or 18% egg for 6 or 9 days resulted in liver weights lower than those obtained on 6% egg. Pale livers suggestive of fatty infiltration were observed in animals refed with 0% egg and 3% egg, but not with higher levels of protein.

Protein nutrition has been shown to have a marked effect on the composition of hepatic cells (Munro and Clark, 1960, and Muramatsu and Ashida, 1962). Since nucleic acids of the cell are involved in protein synthesis, hepatic RNA and DNA and the ratio of RNA to DNA were determined and used to evaluate the diets in this study.

Total hepatic RNA increased as dietary egg protein was raised to 9%, but no further increments in RNA content were observed by feeding 18% egg. Repletion for 6 days produced slightly higher total hepatic RNA than 5 days of feeding except in rats given no protein. Therefore, protein biosynthesis was intensified by raising egg protein to 9%, but no further enhancement was obtained by intakes twice as high.

Hepatic RNA concentration was highest with 18% egg repletion while lower values were found at lower protein levels.

Lengthening the repletion period from 5 to 6 days resulted in slightly increased hepatic RNA concentrations.

Both total hepatic DNA and hepatic DNA concentration were highest in rats fed 18% egg protein. Animals repleted with 3%, 6%, or 9% egg had similar total hepatic DNA values which were higher than that of animals repleted with 0% egg. However, DNA concentration was lower in groups repleted with 6% egg for 5 days or 9% egg for 6 days than in groups refed 0%, 3%, and 9% egg for 5 or 6 days, respectively. These data indicate that total hepatic DNA was relatively constant and independent of nitrogen intake between the 3% and 9% egg protein levels, but very low or high protein intakes resulted in significant decreases or increases, respectively, in DNA content. Several investigators (Williams, 1961; Umaña, 1965; and Kenney et al., 1968) have reported increased hepatic DNA concentrations in rats fed protein-deficient diets. Svoboda et al., (1966) considered the apparent increase in DNA concentration to reflect a more rapid loss of cellular constituents other than DNA. Loss of total hepatic DNA in protein-calorie restricted rats subsequently refed the protein-deficient diet ad libitum perhaps reflects the restricted caloric value of the diet during depletion since this observation has not been reported previously.

RNA/DNA ratios were determined in this study since this parameter has often been used to estimate changes in RNA

concentration at the cellular level. Protein repletion produced significantly higher RNA/DNA ratios than repletion with 0% egg protein, but no changes in RNA/DNA ratios were noted by raising the level of egg protein from 3% to 9% in the 5-day repletion period. However, during the 6-day repletion period 9% egg produced a significantly higher RNA/DNA ratio, 2.69, than did 3% egg, 2.35, or 6% egg, 2.38, while lower ratios were found with 18% egg repletion, 2.00 and 2.12, for 6 and 9 days respectively (Table 7).

The increase in the RNA/DNA ratio with protein repletion suggested that protein biosynthesis was stimulated by incorporation of protein into the diet. However, protein biosynthesis appeared to be independent of the level of nitrogen intake from egg protein during 5 days of repletion since RNA/DNA ratios were not significantly different among the egg-containing experimental diets. Protein repletion for an additional day, i.e., for a total of 6 days, seemed to enhance protein biosynthesis, as RNA/DNA ratios were slightly higher after 6 days repletion than after 5 days, e.g., 2.38 vs. 2.10 for 3% egg. Feeding 9% egg protein for 6 days appeared to stimulate protein biosynthesis maximally since the RNA/DNA ratio for this group was significantly higher than those of groups fed 3% or 6% egg. The lower RNA/DNA ratios observed after feeding 18% egg were probably due to an increase in total hepatic DNA because the total hepatic RNA content was similar for groups fed both 9%

egg and 18% egg for 6 days.

Splenic RNA and DNA and the Immune Response

Spleen and serum immunoglobulins were examined in immunized rats repleted with 0% to 18% egg protein in an attempt to characterize quantitatively the relationship of size and composition of the spleen to its production of immunoglobulins.

Spleen weights were significantly reduced during protein depletion; however, weights tended to increase as the amount of egg protein was raised in the diet. An exception was the rats fed 6% egg who had slightly greater mean spleen weights than rats given 9% egg on the 5th day of the primary immune response, which was also the 5th day of repletion.

Total splenic RNA tended to increase with increases in dietary egg protein, which suggest that protein biosynthesis was enhanced by increased nitrogen intakes. Rats refed 0% egg, 3% egg, or 6% egg had similar amounts of splenic RNA for the 5th and 6th days of the primary immune response while total RNA was greater on day 6 than day 5 for groups refed 9% egg, 5.40 mg vs. 4.57 mg. These findings suggested that protein biosynthesis did not differ between day 5 and 6 of the immune response for rats refed 0%, 3%, or 6% egg protein, but increased somewhat on day 6 for animals given 9% egg protein. Total RNA increased only slightly when 18% egg was fed for 6 days, to 5.73 mg, but decreased to the 6% egg value, 4.61, when reple-

tion was continued for 9 days. Thus, protein biosynthesis was enhanced slightly by repletion with 18% egg for 6 days, but with 9 days of repletion no further stimulating effect was apparent; indeed approximately 20% less RNA was present in the spleen on day 9 than on day 6.

Five days after immunization, splenic RNA concentration of protein-repleted animals was significantly greater than that of rats refed 0% egg; however, no differences were found by varying the level of egg protein from 3% to 9% (Figure 1). On the 6th day of immunization RNA concentration increased progressively as egg protein was raised from 3% to 18%, from 5.13 mg to 6.24 mg/g spleen (Figure 2). However, repletion with 18% egg for 9 days resulted in a slight decrease in RNA concentration to 5.96 mg/g spleen. These findings suggest that although the incorporation of protein in the repletion diet enhanced protein biosynthesis, cellular content of RNA did not increase after raising the amount of nitrogen intake from egg protein until the 6th day of the immune response, which was also the 6th day of repletion and the day of peak antibody production.

Total splenic DNA on both day 5 and day 6 of the immune response increased as dietary egg protein was raised from 0% to 9% (Figures 1 and 2). Further elevation to 18% egg on day 6 resulted in a decrease in total DNA. This decrease became more pronounced by lengthening the repletion period to 9 days. Thus total splenic DNA appeared to be dependent on the amount

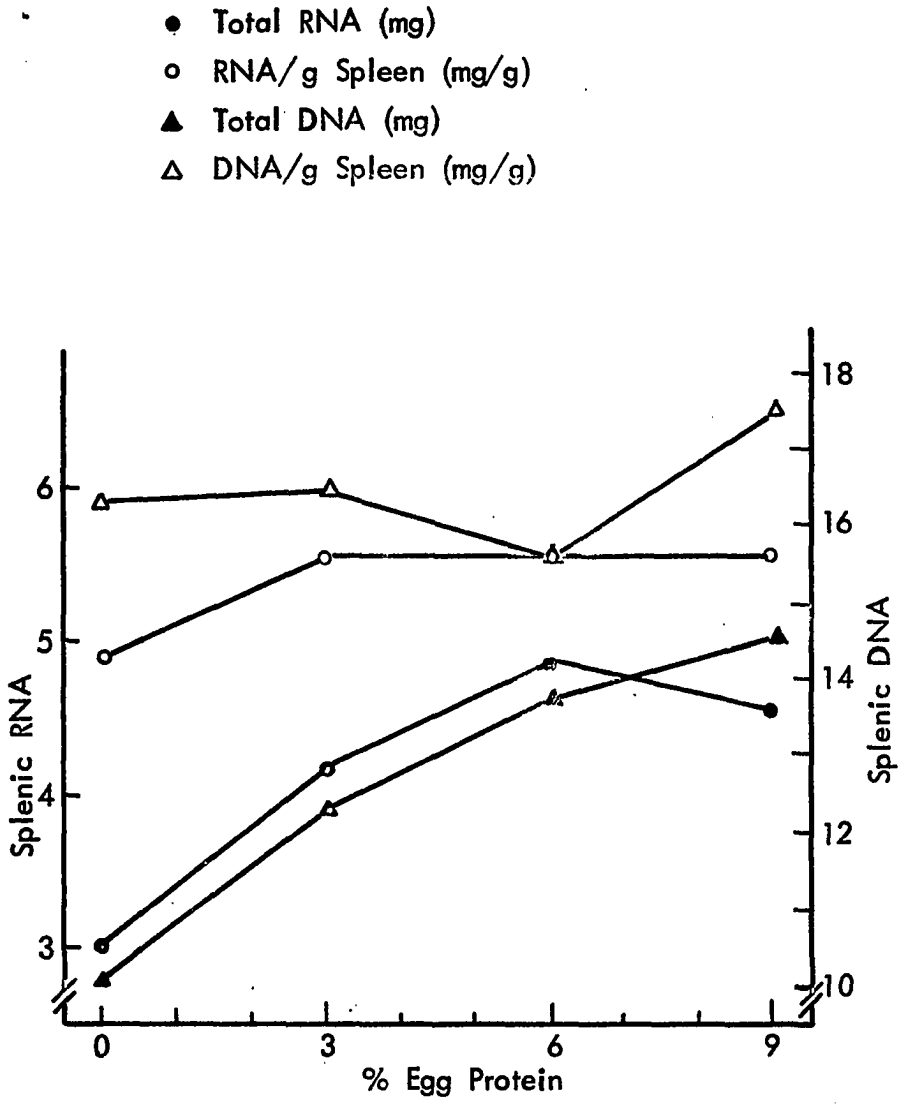

Figure 1. Splenic RNA and DNA vs. amount of dietary egg protein for rats on the 5th day of the immune response

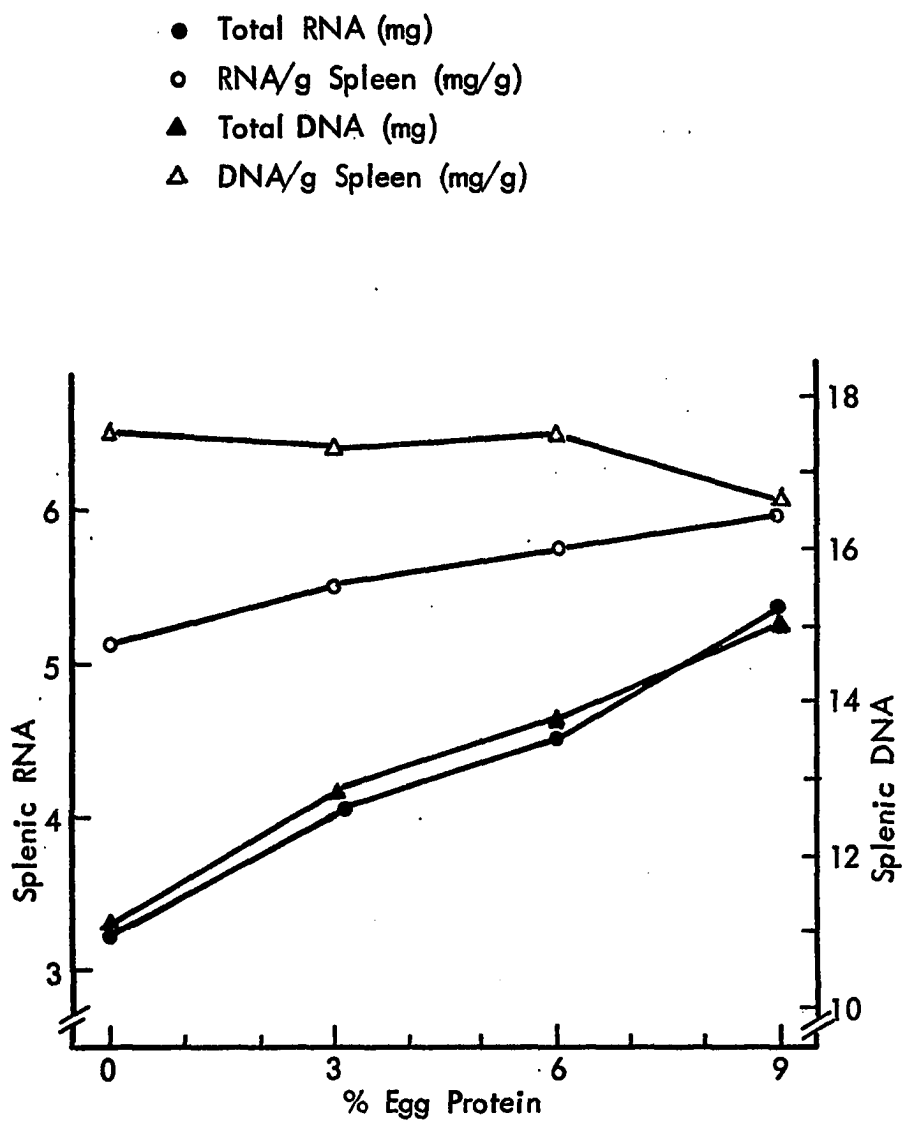

Figure 2. Splenic RNA and DNA vs. amount of dietary egg protein for rats on the 6th day of the immune response

of nitrogen intake at low or normal protein intakes, but not at high protein intakes.

DNA concentration in the spleen was independent of nitrogen intake on the 5th day of the immune response (Figure 1). Increasing the level of egg protein to 9% or 18% produced a slight decrease in DNA concentration 6 days after immunization (Figure 2). Therefore, on the 5th day of the immune response, there had been simultaneous increases in splenic weight and total DNA, which were proportional for all groups. However, 6 days after immunization in groups fed 9% or 18% egg the increase in splenic weight must have been due to constituents other than DNA such as RNA or protein.

The increase in the RNA/DNA ratio when protein was included in the repletion regime suggested that protein biosynthesis in the spleen was stimulated by dietary protein. On the 5th day of the immune response, RNA/DNA ratios in protein-repleted rats were slightly higher in groups fed 3% egg, 0.35, or 6% egg, 0.36, than in groups fed 0% egg, 0.31, or 9% egg, 0.33. Six days after immunization the RNA/DNA ratios increased slightly with all increases in dietary nitrogen, suggesting that on the 6th day of the immune response protein biosynthesis was proportional to nitrogen intakes from egg protein.

Circulating antibodies, hemolysins and agglutinins, were significantly reduced in 0% egg-repleted rats when compared with stock-fed animals (Experiment II). Antibody titers were

independent of the nitrogen intake supplied by egg protein. In addition, gamma globulin concentration in the serum did not follow the same pattern of variation as the antibody titers.

Five days after immunization, rats repleted with 3% egg exhibited a significantly higher mean agglutinin titer than groups repleted with 6% and 9% egg and a significantly higher mean hemolysin titer than rats refed 0% egg protein (Figure 3). Serum concentration of gamma globulin was similar for groups refed 3% and 6% egg protein; however, repletion with 3% and 9% egg produced significantly greater mean gamma globulin concentrations than 0% egg repletion.

The splenic nucleic acid values of animals on the 5th day of the immune response suggested that animals repleted with low levels of protein maintained antibody production at the expense of other proteins such as gamma globulin or spleen protein. Rats refed 0% egg exhibited low total amounts splenic RNA and DNA and low concentrations of serum gamma globulin while maintaining antibody titers comparable to those of groups repleted with egg protein (Figures 1 and 3). Repletion with 3% egg produced an increase in both antibody and gamma globulin production which coincided with elevated quantities of splenic RNA and DNA. However, further increases in nitrogen intake from 6% or 9% egg diets decreased antibody production while serum gamma globulin levels remained constant or showed a slight increase. Splenic nucleic acid data suggested that higher nitrogen intakes stimulated the production of proteins

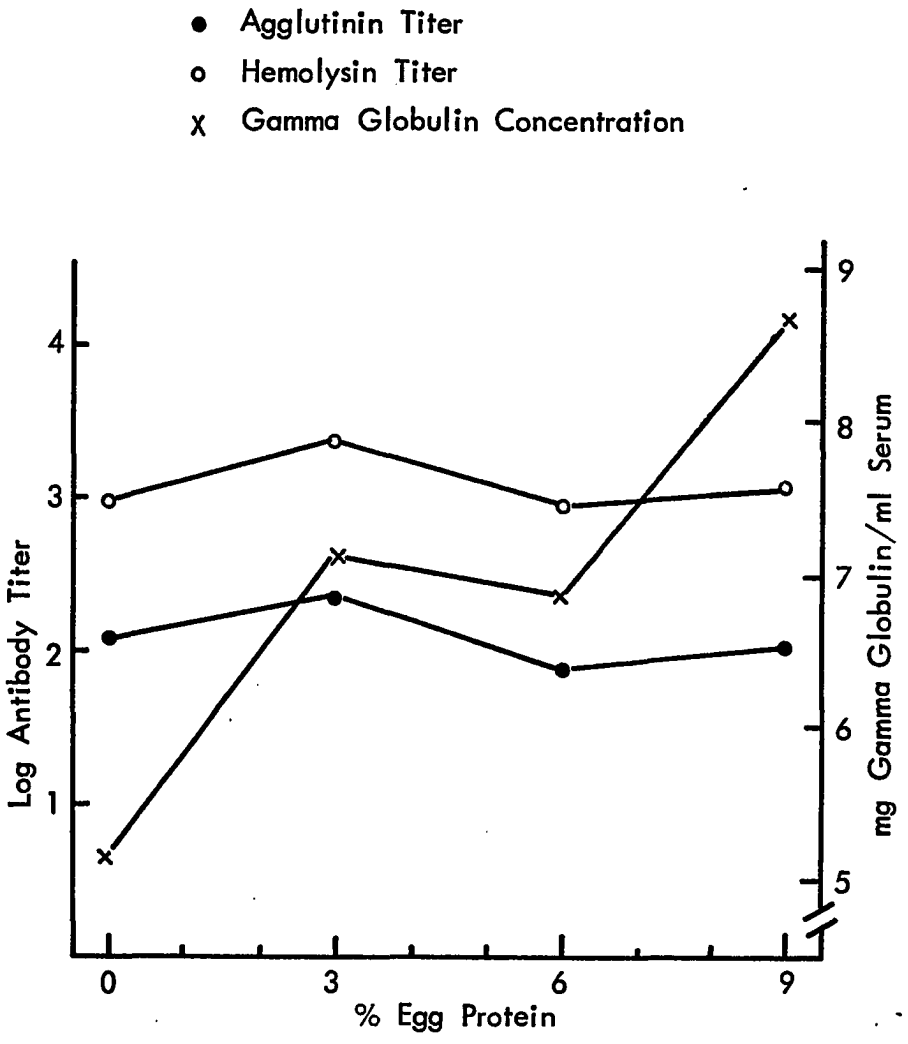

Figure 3. Antibody titers and gamma globulin concentration vs. amount of dietary egg protein for rats on the 5th day of the immune response

other than specific antibody to sheep red blood cells. As dietary egg protein was raised from 0% to 9%, total splenic RNA increased significantly from 3.02 mg to 4.57 mg and total splenic DNA from 10.08 mg to 14.58 mg (Figure 1).

On the 6th day of the immune response antibody titers and gamma globulin content had increased in 0% egg-repleted rats compared with values on the 5th day (Figures 3 and 4); slight elevations in splenic RNA and DNA occurred at the same time. However, 3% egg-repleted animals exhibited somewhat lower antibody titers and a greater reduction in gamma globulin than groups refed with 6% or 9% egg, which remained relatively constant with respect to antibody titers and gamma globulin concentration. Since splenic RNA and DNA content, gamma globulin concentration, and antibody titers in 0% egg-repleted rats were somewhat greater on day 6 than on day 5 of the immune response, it appeared that splenic cellular proliferation, protein biosynthesis and antibody production were more active on the 6th day. However, higher antibody titers and gamma globulin concentrations on day 5 than on day 6 in rats refed 3% egg suggested that splenic protein biosynthesis and antibody production were greater on the 5th day of the immune response than on the 6th day for that group. Splenic DNA content which was greater on day 6 indicated an increase in cellular proliferation over day 5.

Similar splenic RNA content and gamma globulin concentration for day 5 and day 6 of the immune response in rats refed

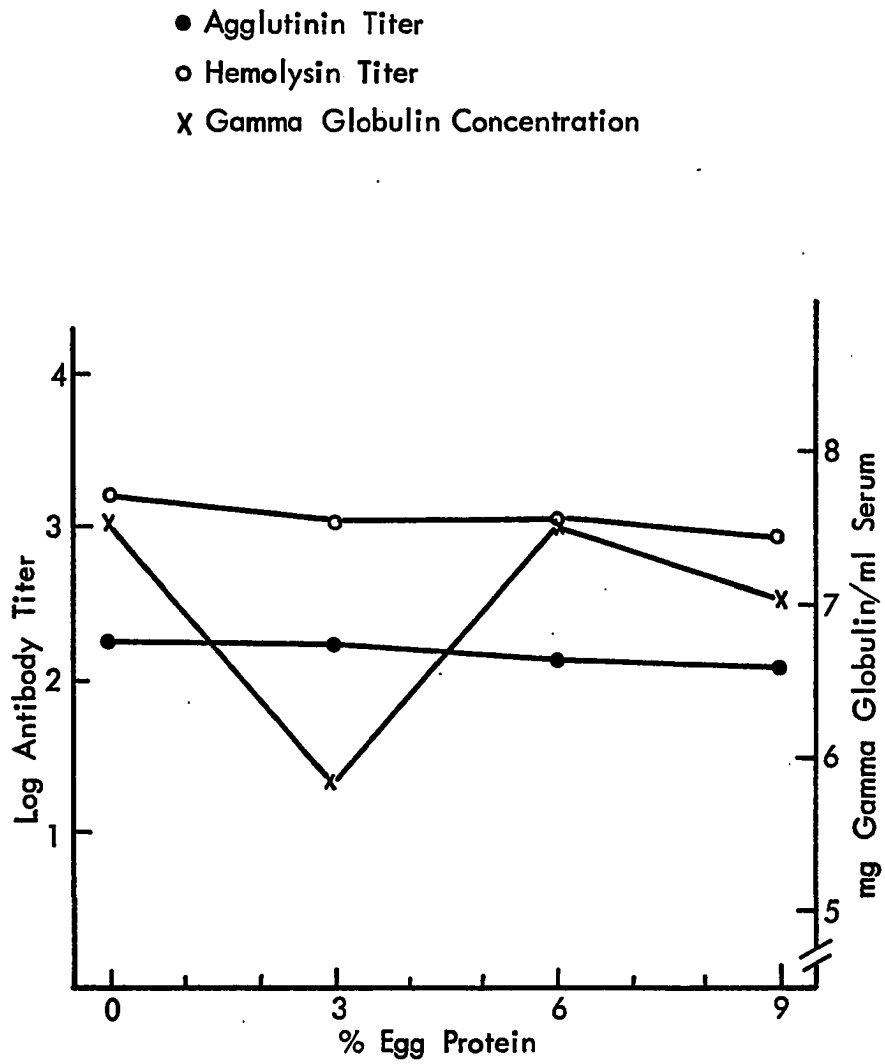

Figure 4. Antibody titers and gamma globulin concentration vs. amount of dietary egg protein for rats on the 6th day of the immune response

6% egg suggested that splenic protein biosynthesis was as active on the 5th day as on the 6th day. However, the antibody response and the number of cells in the spleen (DNA content) had increased on day 6 of the immune response in 6% egg-repleted rats.

Between days 5 and 6 of the immune response, animals repleted with 9% egg protein exhibited a reduction in gamma globulin concentration which coincided with a decrease in splenic DNA concentration, but antibody titers remained relatively constant and a slight increase was noted in splenic RNA. On the 6th day of the immune response, increasing dietary egg protein to 18% seemed to produce fewer spleen cells than the other protein levels did; gamma globulin concentrations were similar to that observed in rats refed 3% egg. However, splenic protein biosynthesis and antibody production remained at relatively high levels.

Radioactivity Measurements

Experiments II and III of this study utilized ^{14}C-lysine to determine in immune rats the effects of various levels of egg protein on plasma protein metabolism with emphasis on the antibody and gamma globulin fractions.

Following intravenous injection of a labeled amino acid, the tracer is distributed throughout the intravascular system within a few minutes. For example, determination of plasma

radioactivity and hence, the dilution of the dose, within 5 minutes after tracer injection, has been used to estimate plasma volume (Schultze and Heremans, 1966). The tracer dose rapidly leaves the circulation and has been found to be incorporated into intracellular proteins immediately (Peters, 1962, and Humphrey and Sulitzeanu, 1958). Labeled proteins have been observed in the blood 18 to 20 minutes after tracer injection and thereafter nearly all plasma radioactivity is considered to be associated with plasma proteins and not with free amino acids (Peters, 1962; Humphrey and Sulitzeanu, 1958; and Schultze and Heremans, 1966). Therefore, in this study plasma radioactivity, 3 hours and 24 hours after tracer injection, was assumed to represent radioactive plasma proteins and not labeled free amino acids.

Changes in radioactivity of serum and excreta between 3 hours and 24 hours after tracer injection and the calculated half-lives of total serum proteins indicated that in rats fed 18% egg protein, or the stock diet, the turnover rate of total plasma proteins was very rapid, with "half-lives" of 1.3 to 1.6 days. Turnover rate appeared to increase with elevation in dietary protein from 9% to 18%. Other studies have shown that changes in total plasma radioactivity paralleled those observed for serum albumin; hence, total plasma radioactivity changes have often been considered to represent those of the albumin fraction which composes the bulk of plasma proteins

(Schultze and Heremans, 1966). In this study it appeared that serum albumin turned over very rapidly in rats fed 18% egg protein and a mixture of proteins as in the stock diet. Hepatic nucleic acid data support this conclusion because high concentrations of hepatic RNA indicated protein biosynthesis was enhanced at high nitrogen intakes.

When rats were repleted with 0% egg or 3% egg protein in Experiment III, turnover of plasma proteins appeared to be 1/2 as rapid as observed at 18% egg or higher protein levels. Serum radioactivity data indicated that animals repleted with 0% egg in Experiment II had "half-lives" for total serum proteins comparable to those of similarly treated rats in Experiment III. Measurements of serum radioactivity indicated that turnover rates of serum proteins in rats refed 9% egg and 6% egg were similar and intermediate between those of rats refed 0% and 3% egg and those receiving 18% egg protein and stock rations. Serum radioactivity data coupled with other observations, suggested that with respect to total plasma protein metabolism diets supplying 6% and 9% egg were adequate or optimal, those containing 3% egg or 0% egg were insufficient and 18% egg or stock diets were excessive in the nitrogen content.

Radioactivity data indicated that rats fed 9% egg had the shortest "half-life" for serum gamma globulin; the "half-life" of total serum proteins for this group was slightly longer than that for gamma globulin. The calculated "half-life" for gamma globulin was approximately four times as long in rats repleted

with 3% egg protein as the 9% egg groups when data for (radioactivity/ml serum) per (dose/100 g body weight) were used. A somewhat longer estimation of "half-life" for gamma globulin was obtained for 6% egg-repleted rats from total radioactivity data.

The groups repleted with 6% egg exhibited a "half-life" for gamma globulin which was comparable to that of 3% egg-repleted rats when estimates were based on data for gamma globulin radioactivity per ml serum adjusted for tracer dosage per 100 g body weight.

Gamma globulin "half-life" could not be estimated for rats refed 0% egg since gamma globulin radioactivity increased between 3 and 24 hours after tracer injection.

Data for specific antibody to sheep red blood cells based on total radioactivity and radioactivity/ml serum adjusted for tracer dosage per 100 g body weight showed that rats fed both 0% and 3% egg had specific antibody with the shortest "half-lives", approximately 2 days. Slightly longer "half-lives" for specific antibody were found for groups repleted with 9% egg protein, 3 to 4 days, while antibody "half-lives" could not be determined from these data for rats fed 6% egg. When radioactivity data for isolated antibody were corrected for the per cent recovery of specific antibody during the isolation procedure, somewhat different "half-lives" were obtained for the repleted groups in Experiment III. Estimated "half-lives"

were 1 day for 0% egg repletion, 1.5 days with 3% egg and 3.2 days with 6% egg. Antibody "half-life" could not be determined from this data for rats refed 9% egg. The longer antibody "half-lives" observed at the higher levels of egg protein might have been due to a greater percentage of 7S rather than 19S antibody present in these animals. Rats refed 0% or 3% egg may have had mainly 19S antibody which is produced initially in the immune response and later followed by 7S antibody which persists for a longer period of time.

Several investigators have shown that dietary protein deprivation produced a reduction in the serum catabolic rate and an increase in the half-life of serum albumin and total serum proteins while gamma globulin metabolism did not appear to be altered significantly (Freeman and Gordon, 1964; Hoffenberg et al., 1966; and Steinbock and Tarver, 1954). When protein was returned to the diet, the replacement rate of serum albumin increased and the half-life of albumin shortened as dietary protein was raised while serum gamma globulin was not affected (Jeffay and Winzler, 1958b). Although serum gamma globulin metabolism has been reported to be relatively independent of nutritional status, the rate of gamma globulin synthesis has been observed to be above normal when protein deficiency was complicated by infection (Cohen and Hansen, 1962). The present study indicated that during the primary

immune response in rats, the level of dietary protein affected the metabolism of serum gamma globulin and specific antibody as well as that of total serum proteins.

SUMMARY

A series of three experiments was conducted to study the immune response and other related parameters in rats fed several levels of egg protein. Adult, male rats, weighing between 459 and 516 g, were depleted on a low protein, calorie-restricted diet for 3 to 6 weeks and refed for approximately one week on diets that varied in quantity of egg protein, 3% to 18%. Groups fed a stock ration throughout or a 0% egg diet during repletion served as controls.

One ml of a 2% solution of sheep red blood cells was injected into the caudal vein of the rats either six days prior to autopsy or on the 1st or 3rd day of repletion, in which case they were killed 5 or 6 days later. When ^{14}C-lysine was utilized, it was injected into the caudal vein 5 days after immunization and 3 or 24 hours prior to killing the animal. Blood was collected 3 and/or 24 hours after injection of tracer for determination of agglutinin, hemolysin, gamma globulin concentration, and half-lives of total serum proteins, gamma globulin and specific antibody to sheep red blood cells. Livers and spleens were analyzed for ribonucleic acid and deoxyribonucleic acid.

During depletion rats lost 20% to 25% of their initial body weight and repletion with 0% to 18% egg protein produced increases in body weight as well as liver and spleen weights. Although raising dietary egg protein from 0% to 6% produced

concomitant increases in weight gains, raising egg protein to 9% or 18% did not always produce further increments in organ or body weight gains.

Comparison of daily amino acid intakes from egg protein to minimum daily requirements for repletion and maintenance indicated that isoleucine was the first limiting amino acid followed closely by lysine. Roughly 1/3 of the requirement for the essential amino acids was supplied by the 3% egg diet, 1/2 by 6% egg, 3/4 by 9% egg, while 18% egg provided an excess of all essential amino acids.

Total hepatic RNA increased as dietary egg protein was raised from 0% to 9%; however, feeding 18% egg produced no further increments. Total DNA content of the liver appeared to be independent of nitrogen intake between 3% and 9% egg protein, but 0% egg or 18% egg were associated with a significant decrease or increase, respectively, in DNA content. Hepatic RNA/DNA ratios were significantly increased with protein repletion using diets containing 3, 6, or 9% egg; however, only after repletion for 6 days were the ratios significantly higher for groups refed 9% egg than for those fed 3% or 6% egg. Repletion with 18% egg for 6 or 9 days resulted in RNA/DNA ratios similar to those found with 0% egg repletion.

Five days after immunization, which was also the 5th day of repletion, incorporation of protein into the repletion diet produced an increase in splenic total RNA, total DNA, and RNA

relative to DNA. However, no significant differences in total RNA and RNA/DNA ratios and only a slight progressive increase in total DNA were observed among groups refed 3%, 6%, and 9% egg protein.

On the 6th day of the immune response, which was also the 6th day of repletion, total RNA, total DNA, and RNA relative to DNA in the spleen tended to rise with increases in dietary egg protein from 0% to 9%. Repletion with 18% egg protein for 6 days produced further increments in total RNA and in the RNA/DNA ratio while 18% egg for 9 days increased the RNA/DNA ratio and decreased total RNA and total DNA in the spleen relative to 9% egg.

Agglutinin and hemolysin titers were significantly reduced in rats repleted with 0% egg protein when compared with stock-fed animals; however, antibody titers, although variable, did not appear to be dependent on the level of nitrogen intake as supplied by egg protein on either the 5th or 6th day of the immune response. In addition, serum gamma globulin concentrations also varied but not in the same manner as antibody titers. Five or six days after immunization, antibody titers of rats repleted with low levels of egg protein were either comparable to or higher than titers of rats refed greater amounts of protein. Gamma globulin concentration was elevated with the incorporation of egg protein into the diet on day 5 of the immune response and of repletion; however, no significant

differences could be shown among groups fed 0% to 18% egg on the sixth day.

Total serum radioactivity data suggested that the "half-life" of total serum proteins decreased as dietary protein was increased from 0% egg to 18% egg or 25% supplied as a mixture of vegetable and animal proteins. Since changes in total serum radioactivity are often considered to represent those of the albumin fraction which composes the bulk of serum protein, the "half-life" calculated for total serum protein may represent generally that of the albumin fraction.

Radioactivity data indicated that the "half-life" of serum gamma globulin in rats repleted with 9% egg protein was very short, about 2 days. "Half-lives" for gamma globulin in animals repleted with 3% or 6% egg appeared to be approximately four times as long as that of rats refed 9% egg. Since gamma globulin radioactivity in rats refed 0% egg increased between 3 and 24 hours after tracer injection, the "half-life" of gamma globulin could not be estimated.

"Half-lives" for specific antibody to sheep red blood cells in groups refed low levels of protein, 0% and 3% egg, were about 1 to 2 days and appeared to be 1/2 to 1/3 as long as antibody half-lives in groups refed higher levels of protein, 6% and 9% egg, approximately 3 to 5 days. However, the antibody measured in rats refed 6% or 9% egg may have been mainly 7S which persists longer after immunization than 19S antibody

which may have been predominant in rats refed 0% or 3% egg.

Incorporation of protein in the repletion diet improved the response of rats for most parameters measured, except for circulating antibodies. The immune response of rats, as assessed by antibody titers was not adversely affected by dietary protein of very low levels, 0% and 3% egg.

This study indicated that egg protein was of no benefit when 9% was fed, though 18% increased antibody titers to levels intermediate between those observed for depleted and normal rats.

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