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THE EFFECT OF THE QUALITY AND QUANTITY OF VARIOUS DIETARY PROTEINS ON THE IMMUNE RESPONSE OF RATS

by

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LIST OF ABBREVIATIONS

Srbc	- sheep red blood cells
NER	- nitrogen efficiency ratio
RNA	- ribonucleic acid
DNA	- deoxyribonucleic acid
AFC	- antibody-forming cells, plaque-forming cells
DD	- low nitrogen diet or depletion diet
R6	- 6% rice protein
C6	- 6% corn protein
CF10	- 6% corn protein, 4% fish protein
RF10	- 6% rice protein, 4% fish protein
RCE10	- 3% rice protein, 3% corn protein, 4% egg protein
R5	- 5% rice protein
RM5	- 3½% rice protein, 1½% mung bean protein
RAA6½	- 5% rice protein, 1½% isolated amino acid mixture
RAA7½	- 5% rice protein, 2.5% isolated amino acid mixture
RM7½	- 5% rice protein, 2.5% mung bean protein
Scly	- Fed stock ration continuously for 6 weeks; not depleted
Scol	- Taken from the stock colony six days prior to autopsy
Sr	- Depleted and then refed a stock ration
Sni	- Fed stock ration and not immunized
PCA	- Perchloric acid
gm	- gram or grams

Sc - Fed a stock ration and injected 1 ml of a 1:10 solution of Salmonella O antigen

Sd - Fed a stock ration and injected 1 ml of a 1:100 solution of Salmonella O antigen

INTRODUCTION

Undernutrition is one of the most serious health problems of the world today. It is especially severe in countries where food supply cannot meet the demands of the ever-increasing population. Although it may not be listed frequently as a cause of death in children, it is one of the factors that contribute to high morbidity and high mortality rates among infants and pre-school children.

Protein-calorie lack in the diet, particularly in vulnerable age groups, leads to the serious nutritional deficiency diseases--kwashiorkor and marasmus. In areas where animal protein is scarce, where it is only within the reach of the well-to-do, where religious practices forbid its use, vegetable proteins which are cheap and abundant have been the proteins of choice.

Studies have shown that a mixture of proteins may be better than a single source (Harper et al., 1955; Bressani and Scrimshaw, 1961).

Various standards for evaluating protein such as nitrogen equilibrium and rate of growth have been reported in the literature (Cannon, 1945). Since hepatic lipid responds to variations in amounts as well as amino acid composition of the dietary nitrogen source, it has also been used as one of the measurements for evaluating dietary protein (Harper, 1958; Olson et al., 1958). Vegetable proteins, which are of lower

biological value than animal proteins, have been evaluated by such standards. However, changes in the protein systems that are important for the proper functioning of vital organs may not always be reflected by these measurements. Evaluating proteins by these parameters may not reflect the consequences of feeding such mixtures on the proper functioning of sites of protein synthesis such as the liver and the spleen.

It is recognized that there is a close relationship between the onset and course of many infectious diseases and malnutrition, but such relationship is not well understood (Scrimshaw, 1966; Dubos and Schaedler, 1959). Axelrod and Pruzansky (1955) have established the need for certain vitamins for antibody synthesis, whereas the role of dietary protein has been more difficult to elucidate. Hence, there is a need to study the basic relationship of antibody formation to protein nutrition in order to clarify seemingly contradictory reports in the literature.

World food surveys (FAO, 1964; Graham, 1965) show that almost half of the world's total protein supply is furnished by grains. Cereals such as rice and corn are the main sources of calories and protein for many of the economically deprived peoples of the world. More than half of the human race consumes rice as the basic staple in the daily diet. In the Philippines, for example, 75-80% of food energy is supplied by rice or corn alone or in combination with other cereals and/or root crops (Quiogue, 1966). For many, pulses such as mung

beans, furnish some of the necessary proteins.

The enrichment of grains with vitamins and minerals may be practical. However, the addition of amino acids is rather difficult not only from the standpoint of cost and availability but also because of the importance of achieving proper supplementation so that the protein in the complete diet is fully utilized. One way of improving the nutritive value of the rice or corn diet would be by supplementing it with other protein foods abundant in a country. Grains and legumes supplement each other (Phansalkar, et al., 1957; Patwardhan, 1961). In many countries mung beans are grown as a second crop after rice harvest not only for its food value but also for use as a fertilizer.

The typical Filipino diet is described as one of "rice and fish". Studies have shown that fish is an excellent source of protein (Carpenter, et al., 1957; Metta and Metta, 1964). When used as a supplement to rice, corn or wheat diets, it has been found to increase the protein efficiency ratios of such diets (Sure, 1957). Many investigations have been reported on the improvement of various cereal diets supplemented with legumes or fish but the immune response of animals to these diets has not been studied extensively.

Although the Filipino diet is typically one of rice and fish, eggs are also consumed in substantial amounts. Egg protein has been widely accepted as a reference protein, and

its relationship to immune response has been studied previously in this laboratory by Glabais (1946) and Kenney (1963). It is therefore of importance to study the effect of rice and corn alone or in combination with egg, fish or mung beans.

The essential amino acid pattern of the protein source is the most important characteristic from the nutritional viewpoint. One of the methods suggested to produce satisfactory protein foods from incomplete vegetable proteins is by amino acid supplementation. It might not be of much practical value to supplement the diet with essential amino acids at present, but as technology is improved and as food supplies become more limited due to a phenomenal increase in population, it may become feasible and practical to add only those amino acids which would give the best growth and meet other criteria, such as immune response.

To investigate the suitability of protein mixtures for optimal antibody synthesis, agglutinins and hemolysins in the serum and antibody-forming cells in the spleen were measured and related to the dietary proteins studied. Hepatic ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) were also determined and related to various dietary amino acid patterns, since nucleic acids play important roles in protein synthesis and are therefore involved in immune response.

The purposes of this study were 1) to evaluate diets containing rice, corn, fish meal, egg and mung bean proteins from dietary intake records, weight gain, hepatic nitrogen,

lipid, RNA and DNA, 2) to determine the effects of these dietary proteins on immune response and 3) to relate the number of antibody forming cells to the amount of circulating antibodies in rats fed the various diets.

REVIEW OF LITERATURE

The relationship between nutritional status and resistance to infection has been the object of study for many years. Several factors play a role in determining resistance to infection, and one of them is the antigen-antibody reaction or immune process. Studies reported in the literature have shown that individual nutrients such as pantothenic acid, pyridoxine, and amino acids from dietary proteins play important roles in the immune process (Axelrod and Pruzansky, 1955; Wissler et al., 1946). The spleen is one of the tissues that is involved in antibody production. Since the purpose of this study is to relate the effect of quality and quantity of dietary proteins on growth and liver composition to the effect on immune response, the discussion that follows will be limited to the influences of dietary proteins on the production of serum antibodies, specifically agglutinins and hemolysins, on the role of the spleen in antibody formation and on other parameters used in the nutritional evaluation of dietary proteins.

Antibody Formation

The body has a variety of defense mechanisms to prevent infection. One of these protective mechanisms is immune response or the ability to synthesize antibodies. An antibody is a protein of the globulin fraction of blood serum. It is probably formed by specific cells of the lymphoid series which have developed into plasma cells from the lymphoid

tissues, such as lymph nodes, bone marrow and spleen, when stimulated by an antigen. The antibody appears in the circulation in response to a foreign particle or antigen.

Sites for antibody production

Histological changes in the spleen, lymph nodes or bone marrow take place before antibodies are detected in the blood (Sen, et al., 1956; van Furth et al., 1966; Wortiss et al., 1966). Intravenous injection of the antigen initiates antibody formation in the rat spleen (Gunderson et al., 1962) while subcutaneous or local injection initiates antibody production in the lymph nodes (Harris et al., 1948).

Several investigators have studied the role played by the spleen in the formation of antibodies. The lymphoid tissue of the red pulp of the spleen is believed to be responsible for antibody production (Gunderson et al., 1962). The release of antibody followed the appearance of plasma cells which may have come from lymphocytes or reticulum cells (McMaster, 1953). This observation was substantiated by the findings of Schultze (1959) that patients with agammaglobulinemia were not able to form plasma cells and no antibodies were produced after antigenic stimulation. Methods such as localized hemolysis in gel (LHG), otherwise known as the agar-plaque technique, and immunocyto-adherence technique (ICA) have been used in quantitatively detecting antibody-forming cells of the spleen (Wortiss et al., 1966; Jerne et al., 1963; Hege and Cole, 1966a,

1966b; Biozzi et al., 1968). Spleen from immunized animals has been shown to synthesize antibody in vitro (Fishman, 1961).

The spleen is necessary for initiating and maintaining antibody production to primary stimulation and is also necessary for the secondary response (Rowley, 1950; Rowley and Fitch, 1965). When rats were splenectomized after a single injection of 1% sheep red blood cells (Srbc) intravenously, continued antibody production was inhibited. Splenectomy after a first injection but before a second injection resulted in a lowered level of antibody production (Campbell and La Via, 1967). Mice spleen cell suspensions formed the largest number of plaques following intravenous injection, while lymph node cells were somewhat less effective (Friedman, 1964).

Biozzi and co-workers (1968) investigated the immune response of mice by determining the number of antibody-forming cells (AFC) in the spleen and the titers of serum antibodies after immunizing with 10^8 Srbc. They reported that there was a close relationship between the rise in antibody-forming cells, agglutinins and hemolysins during the ascending phase of the immune process. The serum antibody was significantly increased at around the third day; agglutinins rose rapidly, thereafter. However, the decline of agglutinins was slower than that of antibody-forming cells. Hemolysins rose more rapidly than agglutinins but declined faster than agglutinins.

The numbers of antibody-forming cells per spleen and serum hemolysin concentrations in mice were studied by Hege and Cole (1966a). They noted fewer plaque-forming cells and lower serum antibody concentrations after a secondary response than after the primary antigen injection. They also observed that the magnitude of secondary response approached that of the primary response when an adequate time interval elapsed between the primary and secondary antigen injections. It is believed that 19S immunoglobulins appear first on the primary response while 7S immunoglobulin appears later and is also abundant in the secondary response (Hege and Cole, 1966b; Wortiss et al., 1966; Adler, 1965).

The spleen reached a maximum weight at around four days after injections of 5% Srbc antigen (Gunderson et al., 1962). There was a definite increase in neutrophilic granulocytes in the red pulp with increase in weight of the spleen. When pyroninophylic cells in the spleen began to decline, circulating antibody reached its peak, around day 6, indicating a lag between synthesis and detection in plasma.

Cannon and Wissler (1967) concluded that migration of pyroninophilic cells and lymphocytes from the spleen to the lymph nodes and bone marrow during immune response in the rat might be important in dissemination of immunological information.

The spleen is not the only site for antibody formation. Taliaferro and Taliaferro (1950) and Kearney and Halliday (1965) observed in rabbits that the spleen was not necessary for the appearance of antibody-forming cells in the blood. For instance, popliteal lymph node produced antibody-forming cells (Fishman, 1961; Hummeler et al., 1966). Thoward and Dalbow (1965) were able to stimulate the oral mucosa to produce antibodies and concluded that antibody production could take place in the gingival tissue of rats and guinea pigs.

The thymus has been linked to the immune process although it has no significant antibody-forming ability (Harris et al., 1948; Friedman, 1964). It has been suggested that the thymus possesses "prominent immunological potencies" at birth and that in adulthood spleen and lymph nodes take over these processes (Csaba et al., 1964). Miller (1962) concluded from his studies on thymectomized mice that the thymus in the newborn helps the immunologic powers to mature, and that the organ may be essential in adults for reestablishing immunologic activity.

Antibody synthesis

Antibodies are synthesized from free amino acids (Schonheimer et al., 1942). Their synthesis has been shown to follow the mammalian protein synthesis pattern in general (Haurowitz, 1965; Strander, 1966; Schultz, 1959).

Several hypotheses for the mechanism of antibody formation have been offered, none of which has been universally accepted. These proposed mechanisms have been classified by Carpenter (1965) under two principal sets of hypotheses: template hypotheses and selective hypotheses.

> The 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.

> The selective hypotheses postulate that "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 postulated and those that react with 'self' components of the individual are destroyed in the embryonic stage; those that survive until immunologic maturity are the antibody-producing cells of the adult". P. 142.

The nature of antibodies and antibody production seems to be accounted for in the selective hypotheses while the template hypotheses account only for the specificity of antibodies.

Characteristics of antibodies

The presence of antibodies in the blood may be recognized by certain characteristic reactions that take place when serum containing antibody is mixed with its antigen.

Different effects due to the reaction of antibody with its antigen are observable. For example, when immune serum is mixed with cellular antigen, cells clump or agglutinate

and the antibody which caused this reaction is known as an "agglutinin". If lysis of the cells occurs, in the presence of complement, the antibodies are termed "lysins". These reactions may be beneficial or harmful: beneficial if they neutralize the toxicity of the antigen, precipitate the antigen, cause cells to agglutinate or lyse, kill the cellular antigen (bactericidal effect), cause capsules of microorganisms to swell or promote phagocytosis; harmful, if they cause anaphylactic shock (Kabat and Mayer, 1967).

Antibodies may vary in serological specificity and molecular weight, but almost all are gamma globulins in their electrophoretic behavior (Freund, 1953; Carpenter, 1965). These variations in size and reactivity may be due to the participation of different organs in the formation of antibodies, the multiple determinant sites against which antibodies are formed, perhaps the antibody-forming cell mutants, related stimuli and length of exposure to antigen (Carpenter, 1965; Haurowitz, 1965; Adler, 1965; Cannon and Wissler, 1967; Wissler et al., 1957).

Dietary Proteins and Antibody Synthesis

Effects of quantity of dietary protein

Since protein is a major constituent of antibodies, quality and quantity of dietary proteins have been related to antibody production. Some investigators have used the immune response in animals fed various dietary proteins as one of the

parameters to evaluate these proteins (Cannon, 1945; Smith, 1966; Williams, 1967; Glabais, 1946; Kenney, 1963).

Proteins, specifically amino acids, have to be supplied in adequate and balanced amounts for synthesis of antibody. Studies have shown that in prolonged protein depletion, circulating antibody titers are markedly diminished (Glabais, 1946; Trnka, 1956; Cannon et al., 1943; Kenney, 1963; Moroz, 1968).

Cannon (1942) found that ability to form agglutinins to typhoid bacilli was decreased when rabbits were fed a low protein ration. White rats on high protein diets, when given a dose of sheep erythrocytes, were able to produce ten times more hemolysin than those on a low protein diet. The same investigator concluded that repleting the protein reserves could restore the capacity to form antibodies.

Glabais (1946) reported that depleted rats had low hemolysin titers compared to those fed a stock ration of meat, milk and grains. Depleted rabbits infected with pneumococci had lower antibody titers in comparison with the well-nourished controls (Wissler, 1947). In rats fed a 3% casein or a protein-free diet, a fall in OH-agglutinins was observed whereas animals on stock ration or 20% casein diets produced increased amounts of antibodies to typhoid vaccine (Moroz, 1968). Trnka (1956) reported that growing rats fed 7% protein had significantly lower agglutinating antibody titers than those fed a 23% protein diet after an infection with Salmonella paratyphii B.

On the other hand, some studies show that, with high protein levels, circulating antibody titers were low. Kenney (1963) observed lower titers in rats fed 18% casein than in those fed 9%. Trnka (1956) also reported significantly lower agglutinating antibody titers in growing rats fed 43% protein than those fed 23%.

The number of antibody-forming cells in the spleen has been related to the amount of agglutinins and hemolysins circulating in the blood in protein-depleted rats and rats fed a stock ration (Kenney et al., 1968). A decrease in spleen weight, number of cells in the spleen, circulating gamma globulins and specific antibodies was observed in protein-depleted rats. The number of antibody-forming cells seemed to be largely responsible for antibody concentration, and the effect of protein deficiency appeared to be in the decrease in numbers of cells that could form globulins rather than a lowered ability of individual cells to synthesize antibodies.

Effects of protein quality

The quality of protein is another factor that affects rate of antibody formation. When good quality proteins such as beef protein or lactalbumin were fed to depleted rats for seven days, the amounts of antibody produced after antigen stimulation almost equaled those of the controls (Wissler, 1947). Vegetable sources of protein were also capable of restoring the antibody-producing capacity to a considerable degree (Kenney

et al., 1965; Wissler et al., 1946). Protein repletion helped not only in the restoration of antibody producing capacity of tissues but also in regeneration of depleted organs such as the spleen and liver (Kenney et al., 1968; Wissler et al., 1946).

Dietary regimens which may have proven to be best for growth and reproduction may not necessarily be those most favorable for antibody production and, for that matter, resistance to infection. Smith (1966) showed that rats fed corn supplemented with fish had lower hemolysin antibody titers than those fed corn alone although they gained more weight than those on the corn diet. Antibody titers of adult repleted rats were significantly higher when soy alpha protein was fed than when casein or gluten was fed, although casein gave most rapid gain (Kenney, 1963).

When human subjects received milk or egg protein at 20 gms per day, higher tetanus or typhoid antibody titers resulted than in those fed only egg protein. A mixture of egg yolk and skim milk protein fed at 58 or 143 gm per day gave higher antibody titers than did lower amounts of protein (Hodges et al., 1962).

Effects of amino acid supplementation

The relative proportions of various amino acids in the diet are as important a factor as their total amount for antibody production. Kenney et al. (1965) reported that methionine supplementation of a protein-free diet at 0.3% depressed antibody formation but only in the presence of glycine. No apparent difference in antibody concentration was found in growing rats given soy alpha protein supplemented with 0.03% methionine or a combination of 0.03% methionine and 0.04% lysine although growth was increased with supplementation (Williams, 1967). At the level of 0.2% lysine supplementation that Smith (1966) used with corn, antibody titers of young female rats decreased and were lower than those of rats fed corn alone. Schaedler and Dubos (1959) found that supplementing gluten diets with lysine could not correct the infection-enhancing effect of gluten diets.

Ryan (1965) gave daily injections of 40 mg phenylalanine per 100 gm body weight to rats. This inhibited antibody production and allowed for survival of skin grafts. When tyrosine, histidine, glutamate, aspartate or methionine was injected, no effects were observed at the levels given.

Depriving rats of protein for 7 weeks and repleting them with 18% casein or 17% complete amino acid mixture resulted in an increase in the number and size of lymphocytes (Aschkenasy, 1966). When one essential amino acid was omitted, the increase

in number and size was prevented. Mixtures deficient in sulphur-containing amino acids, phenolic amino acids, histidine, threonine or valine particularly reduced the lymphocyte count to well below that at the end of the protein deprivation period.

Supplementation of gluten with lysine and threonine allowed for normal recovery of mice from weight loss due to injection of lipopolysaccharide endotoxin of Eschericha coli (Costello et al., 1963). Without supplementation, a delay in regaining weight loss was observed. Mice that were fed casein also rapidly recovered their lost weight.

Gershoff and co-workers (1968) showed a decreased antibody response with tryptophan- and phenylalanine-deficient diets whereas methionine deficiency did not alter antibody response.

Dietary protein and resistance to infection

The relation between nutrition and infection is not well understood, partly because there are many other factors that play a role in determining the resistance to infection. Antibodies contribute to resistance but no one factor controls the outcome of all infections. The state of immunity depends upon the immune response of the host to various organisms, and a fairly reliable index of immunity is the amount of specific antibodies in circulation. Some studies cited have suggested that a direct relationship existed between lowered resistance to infection and decreased antibody formation (Cannon, 1944;

Wissler, 1947). Others, however, did not find any relationship between antibody formation and diminished resistance to infection (Krebs, 1946; Balch, 1950). Scrimshaw (1966) has discussed the effects of an infectious agent and the presence of protein deficiency in the host. When both of these happen in a host that is not well nourished, synergism may occur. This type of relationship has been commonly seen with bacterial and rickettsial infections and also when intestinal protozoa or helminths were involved. Antagonistic interaction between nutrition and infection has been demonstrated in certain cases of infection due to some systemic viruses and protozoa. If changes in the cell associated with severe protein malnutrition are more damaging to the agent than to the host, then antagonism results between protein malnutrition and the infectious agent. Antagonism is likely to happen when an infectious agent has a higher requirement for a particular dietary nutrient than the host.

The genetic make-up of the host is also a factor that affects the reaction of the host to infectious diseases; hence reaction to infectious agents varies from person to person and from species to species.

Britton et al. (1963) fed four-week old chicks a ration containing 5, 10, 15, 20 and 30% protein from soybean meal for two weeks and found that mortality increased with increasing protein in the diet. Less severe cecal lesions were observed

in those chicks fed the 5 and 10% protein diets than those given 15 to 30% protein. Increasing the protein increased the severity of E. tenella infection. There was greater mortality in chicks fed a 30% protein diet when inoculated with S. gallinarum and Newcastle disease virus than those given a 15% protein diet (Smith and Chubb, 1957) while more chicks innoculated with Escherica coli died on a 15% protein intake than on 30% protein (Hill and Garen, 1961).

In a study by Squibb and Grun (1966), the high mortality rate observed in birds that were fed high protein (41.3%) was not due to lowered antibody response. They concluded that, except for extremely deficient diets, dietary proteins had little effect on antibody formation.

Mice have also been used to study the relationship of diet to infection. Schaedler and Dubos (1959) found that mice fed 15 or 20% protein were less susceptible to tuberculosis than those fed 5% casein. When wheat gluten or soybean alpha protein was given, even at 15 or 20% protein, susceptibility to infection developed. When mice were fed soybean and rice flour at 15% of a diet, designed to provide an amino acid pattern similar to that of casein, a satisfactory resistance to bacterial infection was exhibited.

Antibodies aid in phagocytosis and have bactericidal effect which helps in resistance to infection. This aspect in relation to diet has been studied by Guggenheim and Buechler (1948) who investigated the effect of quantitative and

qualitative protein deficiency on bactericidal and phagocytic properties of peritoneal fluid of rats. They fed casein as 3, 6, 9, and 18% of the diet to rats. There was a significant decrease in phagocytic activity in rats fed the 3% protein diet. At the 3, 6 and 9% levels, bactericidal power of the peritoneal fluid was markedly diminished. Rats fed diets containing 9% egg and meat protein had similar bactericidal and phagocytic activity to rats fed diets containing 18% casein. Those on the 9% soy protein and 9% maize protein showed similar reactions to those fed 9% casein and 3% casein, respectively. Rats given the 9% peanut protein showed almost complete breakdown of phagocytic activity whereas their bactericidal power was similar to those of rats fed a 6% casein diet. The authors concluded that "the humoral defense mechanism appears to be more sensitive to protein deficiency than the cellular defense mechanism; different food proteins elicit different bactericidic and phagocytic powers". (Guggenheim and Buechler, 1948, p. 139). The growth promoting effect of the respective proteins corresponded to the antibacterial defense-promoting quality of these proteins.

In 1942, Sako presented evidence that protein of poor quality lowered the native resistance of rats to pneumococcal infection. Rats receiving casein had higher resistance to enteritidis infection than those fed either wheat gluten or soybean flour. There are also studies that indicate that amino acid supplementation may or may not result in high resistance to infection.

Albino rats fed casein, zein, zein plus lysine, or zein plus lysine and tryptophan were inoculated with Trypanosoma cruzi by Yaeger and Miller (1963). Mortality was least in rats fed a 20% casein diet or zein supplemented with lysine and tryptophan and was highest in the rats given zein alone or supplemented with lysine only.

In a study by Hill (1966), addition of arginine and methionine to chick rations containing 20% protein did not significantly affect the mortality of chicks to S. gallinarum. Even at higher amounts of protein, addition of methionine did not influence the rate of mortality.

Gray (1963) fed rats with casein and gluten supplemented with methionine or lysine, equivalent to that in casein. There was a higher rate of mortality among rats fed unsupplemented gluten when infected with Bacillus anthracis spores than among those fed the casein diet. Lysine improved the gluten diet and protected the rats from anthrax, but methionine alone did not.

Results from these studies suggest that no single factor determines resistance to infection. Quality and quantity of protein, the balance of amino acids, type of organism, and species involved are some of the factors which contribute to the outcome of infection.

Weight gain and immunological response

Although increases in protein and amino acid intakes within certain limits do increase weight gains, antibody production may or may not be maintained with increases in weight gain. In Smith's (1966) study, the greatest weight gain occurred in animals fed a corn diet where 40% of the protein was supplied by fish. But of the seven groups tested, this group ranked lowest in hemolysin titers and fourth in agglutinin titers. A tendency toward production of higher antibody titers when egg protein was raised from 9 to 18% during repletion of adult protein deficient rats has been observed. However, with casein, the increase in dietary protein depressed hemolysin titers (Kenney, 1963). In the same study, an inverse relationship of body weight gain to hemolysin titers was noted when protein-depleted rats were repleted with diets containing 9% of various proteins. Although weight gains were highest, hemolysin units were lowest with egg diets. Lysine supplementation of wheat gluten increased body weight also, but hemolysin titers did not increase appreciably with the improvement of the amino acid pattern. When soy was supplemented with methionine, repleted rats gained four times as much as those fed unsupplemented soy protein, yet, hemolysin titers decreased. Williams (1967) was not able to demonstrate any change in hemolysin or agglutinin titers when soy protein diets were supplemented with lysine and/or methionine even though there was an increase in weight gains of growing rats.

Dietary Protein and Hepatic Constituents

Since the liver is a major organ involved in protein metabolism, information on changes in hepatic nitrogen, RNA, DNA and lipid content may be useful in the elucidation of the relationship of nutritional status and resistance to infection. Because the purpose of this study is to relate changes in liver composition to immune responses, only references pertinent to the study were included in this review. There are numerous investigations dealing with the nutritive value of the particular proteins tested in this study, but there are only few reports (to the knowledge of the author) which relate biological values to immune responses. Therefore, a few investigators using similar kinds and amounts of dietary protein are quoted even though their experiments are not concerned with immune responses.

Nitrogen and lipid

Hepatic nitrogen and lipid are used in many instances in evaluating dietary proteins because these liver constituents are affected by quantitative and qualitative changes of protein in the diet. In general, a deficiency in protein will reduce liver nitrogen and may induce accumulation of hepatic lipids. Since hepatic nitrogen tends to increase with an increase in protein intake, an inverse relationship may exist between the level of protein in the diet and hepatic lipid concentration. Furthermore, depending on their amino acid composition,

proteins vary in their effectiveness as lipotropic agents. An imbalance of the amino acid pattern has been shown to induce fatty infiltration of the liver.

Hepatic nitrogen content was high in a group of immunized growing female rats fed a corn diet with 40% of the protein supplied by fish, while low amounts were discerned in the group fed corn as sole source of protein (Smith, 1966). In an investigation by Kenney et al. (1968) using immunized, protein-depleted rats, total liver nitrogen was 63% of control animals while hepatic fat of depleted rats was approximately thrice that of immunized rats given a stock ration. Likewise, rats fed 1.12% nitrogen from polished rice or corn developed fatty livers, but when nitrogen in the diets was increased to 1.60%, liver fat deposition was normal (Ogura et al., 1962). Livers of adult rats maintained on 2% rice protein or casein had almost twice as much fat as livers of rats fed a 20% casein diet. Hepatic nitrogen of animals fed the low-protein diet was lower than that of rats receiving the high-protein diet (Srinivasan and Patwardhan, 1955). A stepwise increase of soy protein in diets of weanling rats from inadequate to excessive levels was highly correlated with the percentage of nitrogen in the liver while hepatic lipid decreased (Mar, 1967).

The effect of variations in the amino acid composition of the dietary protein source on fatty infiltration of the liver has been demonstrated repeatedly. For example, fat accumulated to the extent of 10.8% in livers of young rats fed 90% rice

diet whereas with the same amount of corn, only 3.4% was observed (Harper et al., 1955). Supplementation of the rice diet with 0.4% lysine and 0.24% or 0.5% threonine brought down liver lipid to normal values. When the rice diet contained 3% protein from fish meal, hepatic fat was reduced to 4.8%.

When 90% pre-cooked rice diet was fed to weanling rats, lysine supplementation caused fat deposited in the liver to decrease and protein to increase (Rosenberg and Culik, 1957). Similar observations were made by Mar (1967). When soy protein was supplemented with methionine, liver nitrogen rose above levels found with unsupplemented soy protein diets, while liver fat decreased.

The possibility of supplementing cereal diets with seeds or legumes has been investigated and the effect of supplementation on hepatic nitrogen and fat have been used as measures of changes in protein quality. In this laboratory, Nirmala (1964) used rice or rice plus peanut meal in isonitrogenous diets of young male rats. Hepatic nitrogen increased with the inclusion of peanut in the rice diet while hepatic fat decreased.

In a study by Phadnis (1964) fat deposition occurred even when protein from multipurpose food was partially substituted with rice protein. The proteins were fed at 10% level of the diet. Bressani and Scrimshaw (1961) tried different combinations of rice and black beans or corn and black beans and

observed that although maximum growth was noted, liver fat was high in rats given these diets.

Nucleic acids

Dietary proteins have been shown to influence nucleic acid synthesis in the body. One of the systems dependent on nucleic acid synthesis is antibody formation. Therefore, it is possible that dietary protein plays an indirect role in the immune response through maintenance of nucleic acid synthesis.

That RNA is necessary for antibody formation has been demonstrated by Cohen et al. (1965) in vitro. Spleen cells from non-immunized mice were converted to antibody-forming cells by an extract of RNA from spleen of immunized mice. Hepatic RNA of rabbits immunized with Clostridium welchii underwent configurational changes during biosynthesis of new antibody globulins while no changes were noted with DNA (Pal and Dutta, 1963).

Total RNA as well as the RNA/DNA ratio have been used as a measure of the extent of protein biosynthesis. In protein depletion, a decrease in hepatic RNA, but not in DNA content, has been observed (Allison et al., 1962; Campbell and Kosterlitz, 1947; Kosterlitz, 1947; Summers and Fisher, 1962). Furthermore, with variations in protein intake, no significant effect on DNA content of liver nuclei was noted by Campbell and Kosterlitz (1952) and Thomson et al. (1952, 1953). In general DNA per liver cell seemed to be the least variable constituent

of the liver during protein depletion. However, under certain conditions, a tendency may exist towards an increase in DNA during mild or severe protein deficiency. Ely and Ross (1951) found in young, protein-deficient rats that DNA content per nucleus exceeded that of controls maintained on a stock diet. In another report, total DNA content was elevated in protein deficient adult rats (Umaña, 1965). Kenney et al. (1968) also noted higher hepatic DNA content in immunized protein-depleted rats when compared to immunized stock-fed animals.

RNA and DNA are functionally associated with protein biosynthesis and researchers have found it useful to express RNA relative to DNA. In weanling male rats fed 4% casein, RNA/DNA ratios were moderately decreased when compared to controls (Svoboda et al., 1966). On realimentation with 20% casein, the ratio significantly increased above the control level. A rise in RNA/DNA ratio was noted by Banks et al. (1964) in livers of weanling rats fed increasing amounts of protein. A similar effect resulted from an improvement in the nutritive value of the dietary protein, except when wheat gluten was supplemented with lysine. A relatively high hepatic RNA/DNA ratio has also been observed by Allison et al. (1962) with proteins of high biological value. Egg as the source of protein produced a high RNA/DNA ratio, while cottonseed flour and wheat gluten were associated with relatively low ratios. Mar (1967) noted a significant rise of RNA/DNA ratio in growing rats when methionine-supplemented soy protein was raised from 15% to

22.5% in the diet. Kenney et al. (1968) reported a decrease in RNA/DNA ratio in depleted, immunized adult rats as compared to immunized animals fed a stock ration.

Numerous other investigations on the biological values of the dietary proteins used in the present study are reported in the literature but there is a paucity of data relating these dietary proteins to immune response in rats. In the present study, serum agglutinins and hemolysins, antibody-forming cells of the spleen and changes in nitrogen, lipid, RNA and DNA of the liver as related to immunological response were investigated in rats fed rice and/or corn, in combination with egg, fish, mung beans or crystalline amino acids.

METHODS AND PROCEDURE

General Plan

The study is composed of four experiments -- I, II, III and IV. Adult male rats were depleted for 3 or 4 weeks on a nitrogen-low diet and repleted for 2 or 3 weeks on diets that varied in quality and quantity of dietary protein. Control groups were fed a stock ration or were fed a depletion diet throughout the experiment.

Immunization was carried out by injecting an antigen on the 8th day of repletion. The animals were killed six or fourteen days later. Blood was collected on the sixth day after immunization and, where indicated, on the 10th and 14th days. Livers were frozen for analyses at a later date. Spleens were asceptically removed and prepared for analysis on the same day. A non-immune rat was killed on each autopsy day. Table 1 gives a summary of the experimental plan.

Experiment I

The first experiment was designed to determine how depletion, immunization and a stock diet affect growth and immune response of rats. Similar groups served as controls for the other three experiments.

Adult male rats of the Wistar strain born and raised in the stock colony of the Iowa State University Food and Nutrition laboratory were used in this series. They weighed between 480 and 525 grams. One group of 12 rats (Sr) was placed

Table 1. Summary of experimental plan

Experiment	Group	No. of rats per group[a]	Length of depletion weeks	Length of repletion weeks
I	DD	12	4	
	Sr	12	4	2
	Scly, Scol	12		
II	R6, RF10 RCE10, C6, CF10	10	3	3[b]
	Sc	10		
	Sd	10		
IIIa	C6, CF10, RCE10,	10	3	2
IIIb	R6, RF10	10	3	2
IIIb	Sr	10	3	2
IV	R5, RM5, RAA6½, RM7½, RAA7½	12	4	2
	Scol	12		
	Sni	12		
	DD	10	4	

[a]Rats of Wistar strain in Experiments I, II, III from stock colony of Department of Food and Nutrition, Iowa State University. Rats in Experiment IV from Simonsen Laboratories, White Bear, Minnesota.

[b]Actually repleted for 22 days but has been designated as 3 weeks for convenience.

Table 1 (Continued)

Experiment	Group	Supplements	
		Depletion	Repletion
I	DD	vitamin mix	
	Sr	vitamin mix	vitamin mix[c]
	Scly, Scol	5 gms beef, 10 gms carrots and cabbage on alternate days	
II	R6, RF10, RCE10, C6, CF10	vitamin mix	vitamin mix
	Sc		
	Sd		
IIIa	C6, CF10, RCE10	vitamin mix	
IIIb	R6, RF10	vitamin mix	
IIIb	Sr	vitamin mix	5 gms beef, 10 gms carrots and cabbage on alternate days
IV	R5, RM5, RAA6½	vitamin mix	vitamin mix
	RM7½, RAA7½	vitamin mix	vitamin mix
	Scol		
	Sni		
	DD	vitamin mix	

[c]Rat nos. 81, 82, 85 & 86 were on vitamin mix supplementation for 6 weeks, 89, 90, 93, 94, 97, 98 & 101 had vitamin mix for 5 weeks.

Table 1 (Continued)

Experiment	Group	Kind of antigen[d]	Mode of killing	Source of blood on autopsy
I	DD	Srbc	Blow	Tail
	Sr	Srbc	Blow	Tail
	Scly, Scol	Srbc	Blow	Tail
II	R6, RF10, RCE10, C6, CF10	1:10 Salmonella 0	Injection of sodium pentobarbital	Abdominal aorta or heart
	Sc	1:10 Salmonella 0	Injection of sodium pentobarbital	Abdominal aorta or heart
	Sd	1:100 Salmonella 0	Injection of sodium pentobarbital	Abdominal aorta or heart
IIIa	C6, CF10, RCE10	2% Srbc	Blow	Tail
IIIb	R6, RF10	2% Srbc	Blow	Tail
IIIb	Sr	2% Srbc	Blow	Tail
IV	R5, RM5, RAA6½, RM7½, RAA7½	2% Srbc	Blow	Tail
	Scol			
	Sni			
	DD	2% Srbc	Blow	Tail

[d]Srbc injected 6 days, Salmonella 0 injected 14 days prior to autopsy.

on a nitrogen-low diet for four weeks and repleted on unsupplemented stock ration (Table 7) for two weeks. Another group of 12 rats (Scly) of similar age and weight was transferred from the stock colony and fed the stock diet continuously for six weeks. In addition, supplements of meat balls, carrots and cabbage customarily used in the stock colony were offered to this group. Another set (Scol) of 12 was taken from the stock colony and immunized when the rats in the other groups were injected with the antigen. Scol rats were matched with Sr for weight. The fourth group of rats (DD) was depleted for 28 days. After 22 days of depletion, these depleted rats were injected with sheep red blood cells (Srbc) as antigen.

To immunize rats, one ml of 2% Srbc in saline was injected intravenously. Blood for analysis was drawn from the tail before the animals were stunned and exsanguinated from the neck.

The spleen was removed in the shortest possible time, weighed and analyzed for antibody-forming cells. The percent of nucleated cells of the spleen was also determined.

The liver was excised and weighed. Approximately 1/5 to 1/4 of the total was cut off and reweighed. The smaller piece was wrapped in foil, frozen in liquid nitrogen, sealed in a polyethylene bag and stored at -20°C for RNA and DNA analyses later. The larger piece was not frozen in liquid nitrogen but was kept frozen at -20°C for nitrogen and fat analyses.

Experiment II

Rats were obtained from the stock colony. The animals weighed between 362 and 473 gm and were fed a nitrogen-low diet (Table 2) for three weeks, after which they were given a diet containing rice (R6) or corn (C6) alone or in combination with fish (CF10, RF10) or egg (RCE10) for an additional three weeks. Table 2 gives the composition of the diets.

Eight days after the rats were on the repletion diet, one ml of a 1:10 dilution of Salmonella 0 antigen[1] was injected intravenously. Blood was collected on the 6th, 10th and 14th day after the injections. On the 22nd day of repletion, the animals were killed with sodium pentobarbital. Blood was withdrawn from an abdominal artery or from the heart if the artery had collapsed before any blood could be drawn. Agglutinating antibodies were determined on the 6th, 10th and 14th-day sera. The liver was removed, weighed, wrapped in aluminum foil and stored at a temperature of -20°C until analyzed for nitrogen and fat.

Two other groups of rats, fed the stock diet, were taken from the colony and injected with either the same 1:10 dose of antigen (Sc) or a more dilute 1:100 solution of antigen (Sd) on the day that rats on the experimental diets were injected.

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[1]Bacto-Salmonella 0 Antigen Group A Difco Laboratories, Detroit, Michigan.

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

Ingredients	DD	R6	C6	RF10	CF10	RCE10
			gms/100 gms diet			
Cornstarch[a]	83.00					
Rice starch[b]		4.25	31.60	3.30	26.40	12.10
Fat[c]	10.00	5.00	2.60	4.40	2.30	3.70
Hawk Oser salt mix[a]	4.00	4.00	4.00	4.00	4.00	4.00
Non-nutritive fiber[a]	2.00	1.75	0.80	1.80	0.80	1.30
Sodium chloride	1.00					
Rice[a]		85.00		81.00		42.00
Corn[a]			61.00		61.00	31.00
Fish meal[d]				5.50	5.50	
Egg[e]						5.90
Mung beans[f]						
Protein sources, %						
Rice		6		6		3
Corn			6		6	3
Fish meal				4	4	
Whole egg						4
Mung beans						
EAA & non eaa						
% total protein (calculated values)		6	6	10	10	10
% nitrogen (calculated values)		1.01	0.96	1.60	1.60	1.62
% nitrogen (analyzed values)[g]						
Experiment II		0.96	0.95	1.62	1.63	1.63
Experiment III		1.00	0.93	1.62	1.53	1.58

[a]General Biochemical Inc., Chagrin Falls, Ohio.

[b]Matheson, Coleman & Bell, Norwood, Ohio.

[c]Crisco, Procter & Gamble, Cincinnati, Ohio.

[d]Whole ground herring; Doughboy Industries, Inc., Ames, Iowa.

[e]Hexane extracted whole egg powder; General Biochemical Co.

[f]Oklahoma Jumbo type; Johnston Seed Co., Enid, Oklahoma.

[g]Analyzed by Macro-Kjeldahl method.

Table 2 (Continued)

Ingredients	R5	RM5	RAA[h] 6½	RM 7½	RAA[h] 7½
			gms/100 gms diet		
Cornstarch[a]					
Rice starch[b]	13.34	30.31	11.45	3.18	10.73
Fat[c]	9.80	9.75	9.80	9.60	9.70
Hawk Oser salt mix[a]	4.00	4.00	4.00	4.00	4.00
Non-nutritive fiber[a]	1.86	1.54	1.86	1.32	1.79
Sodium chloride					
Rice[a]	71.00	47.00	71.00	71.00	71.00
Corn[a]					
Fish meal[d]					
Egg[e]					
Mung beans[f]		7.40		10.90	
Protein sources, %					
Rice	5	3.3	5	5	5
Corn					
Fish meal					
Whole egg					
Mung beans		1.7		2.5	
EAA & non eaa			1.7		2.5
% total protein (calculated values)	5	5	6.7[i]	7.5	7.5
% nitrogen (calculated values	0.84	0.83	0.10	1.24	1.24
% nitrogen (analyzed values)	0.86	0.84	1.12	1.26	1.29
Experiment II					
Experiment III					

[h]See Table 3 for amino acid mixture.

[i]For convenience this was rounded off to RAA6½.

In a previous experiment in the same laboratory, all animals injected with the 1:10 dilution of Salmonella antigen had survived. However, some of the animals in Experiment II died a few hours after the injection, thus warranting another experiment using a non-toxic antigen -- sheep red blood cells.

Experiment III

The third study was done in two parts: IIIa and IIIb. Animals fed the corn (C6), corn-fish (CF10) and rice-corn-egg (RCE10) diets were classified under IIIa while those on the rice (R6), rice-fish (RF10) and Stock (Sr) diets were grouped under IIIb. The experimental design was identical for both IIIa and IIIb but the data were obtained at two different periods.

Rats in series III were similar in weight to those in Experiment II. They were depleted also for the same length of time but were repleted for only 14 days. The repletion diets for Experiments II and III were exactly alike. The antigen used was sheep red blood cells. One ml of 2% washed Srbc in saline was injected intravenously. Blood was drawn from the tail before each animal was stunned and exsanguinated from the neck. As in Experiment I, the spleen was removed in the shortest possible time, weighed and analyzed for antibody-forming cells (AFC) soon after autopsy. Unlike Experiment I, nucleated cells were not counted in this experiment. The liver was excised after the removal of the spleen, weighed, wrapped in aluminum foil, frozen in liquid nitrogen and sealed

in a polyethylene bag for RNA, DNA, nitrogen and fat determinations at a later date. On each day of autopsy, a non-immune rat was taken from the stock colony and killed.

Experiment IV

Rats of the Wistar strain used in this experiment were obtained from a commercial source[1] as weanlings and allowed to grow until they weighed between 469 and 525 gm before they were placed on a nitrogen-low diet. These animals, except those on the stock diet (Scol and Sni), were depleted over a four-week period and were on the repletion diet for fourteen days. Twelve rats were assigned to each experimental group and 10 rats to the group on the nitrogen-low diet (DD). Essentially, the procedure outlined for Experiment I was followed in this experiment. The diets contained rice (R5) alone or rice with mung beans (RM5, RM7½) or essential and non-essential amino acids (RAA6½,[2] RAA7½) with a ratio of 2 or 3 parts of rice protein to one part of mung bean protein or amino acids (Tables 2 and 3). The diets were equivalent to 5.0, 6.7 and 7.5 per cent protein.

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[1]Simonsen Laboratories, White Bear, Minnesota.

[2]For convenience the protein equivalent of this diet was rounded off to 6½% and referred to as diet RAA6½. (See Table 3).

Table 3. Essential and non-essential amino acids added to diets RAA$6\frac{1}{2}$ and RAA$7\frac{1}{2}$

Essential AA	RAA $6\frac{1}{2}$	RAA $7\frac{1}{2}$
	gms/100 gms diet	
Arginine monohydrochloride[a]	.185	.272
Histidine monohydrochloride	.085	.124
Lysine monohydrochloride	.200	.294
L-Tyrosine	.054	.080
DL-Tryptophan	.012	.018
L-Phenylalanine	.117	.172
L-Cystine	.003	.005
DL-Methionine	.042	.062
DL-Threonine allo-free[b]	.150	.220
DL-Leucine[c]	.148	.218
DL-Isoleucine	.196	.288
DL-Valine	.111	.162
Non-essential amino acid[d]		
Glutamic acid	.253	.372
Glycine	.258	.379
Alanine[e]	.153	.225
Total amino acid	1.967	2.891

[a]General Biochemicals Inc., Chagrin Falls, Ohio; unless otherwise stated.

[b]One half of the amount was computed as utilized by the rat.

[c]Merck and Company Inc., Rahway, New Jersey.

[d]For total mixture 25% non-essential nitrogen came from glutamic acid and alanine and 50% from glycine.

[e]Matheson Scientific Company, Elk Grove Village, Illinois.

Care of animals

The rats were kept in individual wire-meshed cages on racks in an air-conditioned room at 24-26°C. Distilled water and food were given ad libitum. Animals were weighed thrice a week and food intake records were kept. The cages were changed weekly and papers under the cages changed daily. Fresh food was offered each day. Whatever food was not consumed in two days was discarded and a new amount added to the food jars. Vitamin supplements (Table 4) were given daily in separate cups including two drops of cod-liver oil and two drops of alpha-tocopherol solution added to 500 mgs of the vitamin mixture just before feeding.

Composition and preparation of the diets

The composition of the diets is given in Tables 2 and 3. All protein sources were analyzed for nitrogen. Amino acid composition of the mung bean, given by Gonzalez et al. (1964), was the basis for the calculation of the essential amino acids that were added to the diets (Table 3). The amino acid pattern of the mung bean was simulated in two of the diets - RAA6½ and RAA7½. A mixture of three non-essential amino acids (Table 3) was added to bring the protein content of the diet up to 6½ or 7½. At a later date, the amino acid content of each protein source was analyzed by the method of Spackman

Table 4. Vitamin mix given daily to rats

Components	Amount mg/rat/day
Thiamine hydrochloride[a]	.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 to make	500.0
Two drops daily:	
dl-α-tocopherol	0.75
Wesson oil	49.25
Cod liver oil[b]	50.0

[a]General Biochemicals, Chagrin Falls, Ohio.

[b]Squibb Laboratories, Detroit, Michigan.

et al. (1958)[1]. These analyzed values were used in computing the amino acid pattern of the diets and individual amino acid intake of the rats (Tables 5 and 6). Percentages of fiber, fat and starch were kept constant; hence the amounts of them added per 100 gm of the diet varied according to the amounts provided by the protein sources. Fiber, fat and starch content were calculated from published values (Food and Nutrition Research Center, 1964; Watt and Merrill, 1963).

A green variety of mung beans was autoclaved for 30 minutes under 15 lbs pressure in aluminum pans (Esh and Som, 1952). They were air-dried to constant weight, ground through a mill and stored in brown bottles before they were incorporated into the diet. Polished rice and corn were purchased ground and were added raw or without any further treatment. The fish meal from whole ground herring was obtained from a local manufacturer of animal feed. Defatted whole egg was used in the rice-corn-egg diet (RCE10).

The diets were mixed in large batches, usually enough to last for the whole experiment, and stored in brown bottles or polyethylene containers at 4°C until ready for use.

The components of the stock ration are tabulated in Table 7.

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[1]Wisconsin Alumni Research Foundation Laboratory, Madison, Wisconsin.

Table 5. Essential amino acid composition of the diets, mg/100 grams of diet

	Lysine	Phenylalanine and tyrosine	Tryptophan	Methionine and cystine	Threonine	Leucine
R6	64	400	94	134	171	401
C6	93	351	40	160	168	593
RF10	321	628	137	346	317	671
CF10	353	598	88	379	322	881
RCE10	341	709	139	316	368	866
R5	54	334	78	112	143	335
RM5	87	342	77	96	144	348
RAA6½	214	451	90	157	218	483
RAA7½	289	586	96	179	363	553
RM7½	130	512	115	145	216	522

	Isoleucine	Valine	Histidine	Total essential amino acids gm	Total amino acid gm	Essential amino acid per gram total amino acid
R6	190	276	176	1.91	4.68	.41
C6	160	218	187	1.97	4.55	.43
RF10	332	448	263	3.46	7.97	.43
CF10	311	403	282	3.62	8.06	.45
RCE10	391	508	306	3.94	8.69	.45
R5	158	231	147	1.59	3.92	.41
RM5	172	228	145	1.64	4.01	.41
RAA6½	354	342	210	2.52	5.10	.49
RAA7½	446	393	239	3.14	5.83	.54
RM7½	257	342	217	2.46	6.00	.41

Table 6. Non-essential amino acid composition of the diets, mg/100 grams of diet

	Glutamine	Glycine	Alanine	Arginine	Proline	Serine	Aspartic acid	Total non-essential amino acid
R6	918	150	327	364	236	265	524	2.78
C6	726	115	417	195	412	352	361	2.58
RF10	1347	397	546	585	400	400	840	4.51
CF10	1198	370	652	433	587	499	700	4.44
RCE10	1367	274	624	520	486	604	872	4.75
R5	767	125	273	304	197	222	438	2.33
RM5	778	129	255	289	199	227	489	2.37
RAA6½	791	173	297	457	197	222	438	2.58
RAA7½	802	196	309	529	197	222	438	2.69
RM7½	1166	193	382	434	298	340	731	3.54

Table 7. 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 calcuim pantothenate	0.4
Skim milk[j]	10.2
Cottonseed oil[k]	3.6

[a]General Biochemicals, Chagrin Falls, Ohio.

[b]Froning & 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 & Milling Co., Kansas City, Missouri

[f]Hy-Vee, Ames, Iowa.

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

[h]Trace elements:

	gm		gm
KI	.400	$CuSO_4$	2.036
$MnSO_4$	1.580	$CaCO_3$ to make	1000
$K_2Al_2(SO_4)_4$	0.490		

[i]Six gm 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.

Immunization

Animals in Experiment II were injected with Salmonella O antigen diluted 1:10 (groups R6, C6, RF10, CF10, RCE10 and Sc) or 1:100 (group Sd) on the 8th day of repletion. One ml of antigen was introduced through a lateral caudal vein with a 24 gauge needle after the rat was lightly anesthetized with ether. In Experiments I, III and IV, one ml of 2% Srbc was injected intravenously, also on the 8th day of repletion. Sheep red blood cells[1] suspended in Alsever's solution were washed with isotonic sodium chloride solution (0.85%) twice or until a clear supernate was obtained. The cells were washed by gently tapping the tube and centrifuging for five minutes at a speed of 2500 rpm. After the last washing, time of centrifugation was exactly eight minutes. The washed cells were then diluted 1:50 with 0.85% saline solution, giving a 2% suspension.

Blood collection and storage

Blood was collected from the tail on the 6th and 10th day after injection of the antigen for Experiment II. In Experiments I, III and IV, blood was collected only on the 6th day after injection. This was done by wiping the tail with a wad of cotton soaked in xylene and then making a slit diagonally across a caudal artery. Blood was allowed to flow

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[1]Baltimore Biological Laboratory, West Chester, Pa.

freely into a two-ml centrifuge tube and allowed to clot overnight in the refrigerator. The blood was centrifuged for 30 minutes at a speed of 2000 rpm and the serum stored in glass vials or capillary tubes at -20°C. The wound was wiped with cotton and left to heal. If bleeding continued, a piece of adhesive tape was wrapped around the cut.

On the day of autopsy in Experiment II, the 14th day after immunization, as much blood as could be obtained was drawn from an abdominal artery using a 20 ml syringe and a 20 gauge needle. Blood was collected from the heart whenever there was not enough from the artery or when the artery had collapsed before any blood could be drawn. The blood was allowed to clot at room temperature for 1 to 2 hours. It was centrifuged for 15 minutes at 2000 rpm. The serum was transferred to another tube and recentrifuged for 15 minutes. These sera were stored in glass vials at -20°C.

Autopsy

Rats in Experiment I and IV were starved for 6 to 8 hours prior to autopsy, while those in Experiments II and III were starved for approximately five hours before they were killed. On autopsy days (except in Experiment II) a non-immune rat was also killed.

Animals in Experiment II were killed after three weeks of repletion by injecting 1.5 ml sodium pentobarbital[1] intraperitoneally. If the rats had not lost their reflexes after 10 minutes, another 0.5 ml of pentobarbital was given.

An incision was made through the midline to expose the abdominal cavity without cutting the diaphragm. Blood was then drawn from the abdominal aorta.

The liver was removed, drained of blood by wiping on absorbent paper, weighed, frozen until analyzed for hepatic nitrogen and fat. The amount of abdominal fat was noted by 1, 2, 3, 4 for progressive increase in size of fat pads. The lungs were exposed by cutting through the diaphragm and any abnormalities observed were recorded.

In experiments I, III and IV, the rats were given a strong blow with a rubber mallet, their throats cut and the blood was allowed to drain before an abdominal incision was made. Prior to autopsy, blood was obtained from a tail vein as described in the paragraph on blood collection and storage. Unlike the procedure in Experiment II, sterile techniques were observed until the spleen had been excised aseptically. In these experiments, the spleen was removed for antibody-forming cell determination on the same day before the liver was

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

excised. Livers were weighed and frozen until analyzed for hepatic nitrogen, fat, RNA and DNA. The left testis was weighed in Experiments I and IV. Any gross anatomical changes, subjective evaluation of size of fat pads and other observations were recorded.

Immunochemical Measurements

Titration of antibodies

A modified semi-micro method as described by Smith (1966) was used in the titration of agglutinins and hemolysins. The special microtiter[1] equipment consisted of plates with 96 holes, cups or depressions, a loop designed to contain or hold 0.025 ml of serum, and a dropper pipette calibrated to deliver 0.025 ml. Duplicate samples were analyzed at once or a day apart. Whenever titers of duplicates did not agree within $1\frac{1}{2}$ dilutions a sample was reanalyzed.

Agglutinin In Experiment II, rat sera were analyzed by the following procedure. One drop of 0.85% NaCl was added to all holes of the plate. Rat serum was transferred with the loop into the first hole of each row and serial dilutions made until hole number 9 was reached. Hole 10 did not contain any

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

serum. This served as the blank. With the pipette, one drop of Salmonella O antigen, 1:20 dilution, was added to each cup. The whole plate was incubated for 16 hours in an oven set at 55°C. The highest dilution of serum showing definite agglutination was defined to be the endpoint. Readings were made first without tilting the plates; then plates were read again and were a lighter tinge of blue than unreacted cells. When the endpoint was between two dilutions, the average of the two dilutions was taken as the endpoint. The logarithm of the reciprocal of the highest dilution which was considered the endpoint was used in the calculations.

Sera from rats immunized with Srbc were analyzed for agglutinins by a similar procedure. Two drops of buffer solution (Pillimer et al., 1956) of pH 7.4 were added to the first depression of each row. One loop of serum was then added to the buffer. The solution was incubated for 20 minutes at 56°C to inactivate the complement. One drop of buffer was added to each of the remaining cups and serial dilutions were made thereafter from the original 1:3 dilution. A drop of 1.5 (Experiment III) or 2% (Experiments I and IV) Srbc was added to each cup. The suspension was allowed to stand for 1½ hours at room temperature before readings were recorded. The same method as discussed in the immunization procedure was used in the washing of the Srbc except that cells were resuspended in buffer in the final dilution. Again, the last depression

showing definite agglutination was recorded.

Hemolysin Before the rat serum was titrated for hemolysin, the strength of guinea pig complement[1] to be used in the titration was determined. The lyophylized guinea pig complement was rehydrated with reconstituting fluid, and a 1:10 dilution was prepared with $MgCl_2$-saline solution. A drop of buffer was added to the cups of the first two rows of the plate so that 1:2 dilution of complement was obtained. In the first depressions of the 3rd and 4th rows, two drops of buffer were added to make a 1:3 dilution of the complement. The 1:10 diluted complement was added to the first depression of each row with the loop and serial dilutions were made. The last loop was discarded. A drop of 2% Srbc was added to each cup, followed by a drop of 0.2% rabbit anti-serum containing anti-sheep red cell hemolysin[2]. The plate was covered with plastic film wrap, tapped lightly and incubated in a water bath at 37°C for forty minutes. The endpoint was determined by locating the cup where half of the Srbc were lysed. The concentration of complement that produced the endpoint times four was the amount of complement used for the titrations of the rat sera. To avoid making too many serial dilutions, the rat serum was diluted 1:20, 1:50 or 1:100 in a test tube before making

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

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

serial dilutions in the plates. A drop of buffer was added to all the depressions. A loop of diluted rat serum (one sample for every row) was transferred to the first hole and serial dilutions carried through to the last hole. Two per cent Srbc was dropped in each depression and a drop of complement of appropriate concentration, as previously determined, was added to each cup. The plate was covered, tapped lightly and incubated at 37°C in a water bath for 40 minutes.

Agar plaque-technique for antibody-forming cells

The method for recognizing antibody-forming cells (AFC) in the spleen was modified from the technique of Jerne et al., (1963). Basically, the spleen cells from an immunized animal were mixed with the antigen on a shallow agar layer in a petri dish. The specific antibody diffused out from the cells and reacted with the antigen (Srbc) in places immediately surrounding the antibody-releasing cells. The diffusing antibody formed clear zones that could be seen under slight magnification under the microscope and were recognized as hemolytic plaques among non-lysed red cells.

In this study, as soon as the rat's abdomen was opened, the spleen was excised asceptically, placed in a tared petri dish containing approximately 10 ml balanced salt solution[1]

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[1]Hank's solution 10 x (Tenfold conc.) without $NaHCO_3$ for use in tissue culture media; Hyland Laboratories, Los Angeles, California.

and 0.02 ml of sodium heparin[1]. The spleen weight was taken by difference. The spleen cells were suspended in the balanced salt solution by cutting the spleen into minute fragments with a scalpel (Ackroyd, 1964). With the use of a 2 ml syringe, the cells were pressed out of the tiny pieces by drawing the suspension in and out of the syringe at least 30 times. The suspended cells were transferred into a test tube with a disposable transfer pipette, leaving the connective tissue in the petri dish. The petri dish was not rinsed out. The tube containing spleen cells was allowed to stand in the refrigerator for ten minutes. Clumps settled out and the supernatant fluid was poured into a 15 ml centrifuge tube and was centrifuged at a speed of 1500 rpm for ten minutes. This was the first washing. Washing by this procedure was done thrice. On the last washing, tissue culture medium[2] (Eagle, 1959) instead of balanced salt solution was used. The supernatant liquid was discarded and the volume of the packed spleen cells was recorded. Cells were diluted with an equal volume of tissue culture medium. They were kept in an ice bucket in the refrigerator until ready for plating.

A 1:40 dilution was made from the 1:1 suspension of spleen cells with balanced salt solution, making a final dilu-

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[1]Sodium Heparin injection 1000 USP units/ml; Abbott Lab., North Chicago, Illinois.

[2]Minimum essential medium (Eagle) without l-glutamine, serum or non-essential amino acids; Hyland Laboratories, Los Angeles, California.

tion of 1:80. With the use of a hemocytometer, the number of spleen cells was counted under the high power lens of the microscope. The counting procedure was that proposed for red blood cells (Hepler, 1949, p. 34). A total of eighty small squares, 5 groups of 16 in the central ruled area, were counted:

> Cell count x 10 (depth) x 5(1/5 of sq. mm. counted) x 80 (dilution factor) x the total undiluted volume of spleen cells in cmm = cell count for the whole spleen

Total spleen count was necessary for the determination of the volume of the 1:1 suspension of spleen cells that had to be plated. After several trials it was established that approximately 10^8 spleen cells had to be plated to obtain a reasonable number of plaques for this study.

Agar plates had to be prepared at least two days prior to the analyses of the spleen cells so that the agar might lose 1-2 gm of water. A 1.4% agar solution was prepared by dissolving the agar in balanced salt solution and pouring 8 ml into each 60 x 15 mm petri dish[1]. Enough plates were made to last for two weeks. Before plating, any water that had not evaporated from the agar was poured off. The lids were also wiped off aseptically.

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[1]Disposable petri dish; Plastics Division, Falcon B-D Laboratories Inc., Rutherford, New Jersey.

Tissue culture medium, instead of balanced salt solution, was used in the preparation of 0.7% agar for dispersing the spleen cells. Tissue culture medium was heated up to simmering point and the agar added and dissolved. One ml of the solution was transferred to test tubes immersed in a water bath at 45°C to keep the solution in liquid form.

A portion of a 1:1 spleen suspension, containing approximately 10^7 spleen cells was added to one ml of 0.7% agar in tissue culture medium in a 45°C water bath. Immediately following this step, 0.05 ml of 1:1 dilution of washed Srbc was pipetted into the tube. The contents were shaken gently in the water bath and immediately poured into a previously prepared 1.4% agar plate. A thin top layer was formed by gently sliding the plate back and forth until a uniform distribution was obtained. The plates were incubated in a water bath at 37°C for one hour.

After the incubation period, 0.75 ml of a 1:4 dilution of reconstituted guinea pig complement in buffer was spread over the layer of cells and incubated for an additional half hour at 37°C. Complement was poured off and the number of plaques, recognized as clear areas surrounded by unlysed Srbc, was counted under the 10X power lens of the microscope. The number of plaques under five different randomly selected fields was counted twice on each of triplicate plates. The six readings from the three plates were averaged, and a number of plaques proportional to the total per spleen was calculated

from that average multiplied by a factor. This factor was obtained by dividing the total volume of 1:1 suspension of spleen cells by the volume of spleen cells that had been plated.

Certain precautions had to be observed carefully during the process of recognizing antibody-forming cells of the spleen. To be sure there was no contamination, analysis of spleen from a non-immunized rat was carried out simultaneously. All the glassware, scissors and forceps used were sterilized in an autoclave at 15 lbs pressure for 30 minutes. Disposable sterile petri dishes were used for this purpose. The tissue culture medium and the balanced salt solution were purchased as sterile solutions and were always kept cold. The spleen cell suspension and the washed Srbc were kept either in a bucket of ice or in the refrigerator at all times. The centrifuge cups were chilled before the suspension was centrifuged.

Nucleated cell in spleen

From the suspension of spleen cells, duplicate smears were made on microscope slides. The smears were fixed with alcohol. Staining was done at the end of the experiment with Field's stain (Hepler, 1949, p. 69). The slides were dipped in Solution A (medicinal methylene blue) for one second, washed with distilled water and dipped in Solution B (eosin) for another second. Again, the slide was washed with distilled water until a clear wash water was obtained. The slides were

allowed to dry. Non-drying immersion oil was dropped on the feather end of the stained slide and the number of blue-stained cells were counted as nucleated cells. The number of reddish-pink-stained cells were recorded as red blood cells. Two randomly selected fields were counted and record was made for both kinds of cells. The total counts of the cells were added and averaged to determine the percent of white blood cells.

Chemical Analyses

Nitrogen

Livers, whole or in part, were homogenized and brought to a volume of 100 ml with distilled water. Aliquots of each homogenate were analyzed for nitrogen by the macro-Kjeldahl method (Asso. of Official Agricultural Chemists, 1960). Potassium sulphate and mercuric oxide were used as catalysts in the digestion of the homogenate. Powdered zinc was added to the digest to reduce mercuric oxide before distillation (Hiller et al., 1948). Ammonia was collected in 4% boric acid solution containing a mixed indicator of methyl red and methylene blue and titrated with standardized 0.1 N HCl (Sobel et al., 1937).

The same method was used in the analysis of nitrogen in weighed samples of the experimental diets and of the protein sources of the diets.

Fat

A modification of the method of Soderhjelm and Soderhjelm (1949) was used in the extraction of fat from 10 ml aliquots of liver homogenate. Ethyl alcohol, hexane and diethyl ether were added in succession for the extraction of fat in a Mojonnier flask. Extraction was done twice. The extracts were poured into tared weighing bottles, evaporated over a steam bath, and heated for $15\frac{1}{2}$ hours at $80^{o}C$. The bottles were cooled and weighed after four hours. Fat was calculated by difference.

RNA and DNA

Livers that had been frozen in liquid nitrogen were homogenized and brought to 100 ml with cold distilled water. The livers of rats in Experiment IIIb were homogenized a year before RNA and DNA were analyzed. All the other liver samples in Experiment I, IIIa and IV were freshly homogenized from the frozen state before the analyses.

The procedure described by Munro and Fleck (1966) was modified. The homogenate was filtered through two layers of gauze and the filtrate (0.5 to 3 ml aliquots containing approximately 0.11 to 0.15 gm of tissue) was diluted to 5 ml with cold distilled water. The proteins and nucleic acids were precipitated with 2.5 ml of 0.6 N cold perchloric acid (PCA) and allowed to stand for ten minutes before centrifugation. The supernatant liquid was discarded and the precipitate was rinsed twice with 0.2 N PCA. After the second rinse, the

precipitate was drained briefly before 0.3 M KOH was added. The solution was stirred well and incubated for one hour at 37°C. After the incubation period, the tubes were chilled and the protein and DNA precipitated with 1.2 N PCA. The supernate, which contained the hydrolyzed RNA, was transferred to 100 ml volumetric flasks. The precipitate was rinsed twice with 5 ml of 0.2 N PCA and all the rinses were poured into the solumetric flask. Only 4 ml of 0.2 N PCA was added in the second rinse and an additional 10 ml of 0.6 N PCA was added to the flask. The final concentration of the solution was 0.1 N PCA after the solution was brought to volume with distilled water.

To obtain the DNA in soluble form, 7 ml of 0.3 M KOH was added to the precipitate. The mixture was stirred well and allowed to stand overnight for complete solution of DNA. This solution was centrifuged and the supernate was transferred to a 50 ml volumetric flask. Ten ml of 0.3 M KOH was added again to the precipitate and the supernatant liquid transferred to the flask. The solution was brought to volume with distilled water making a final concentration of 0.1 M KOH.

All steps excluding the incubation period and solubilization of DNA were performed in the cold. Two-thirds of the livers in Experiment IV were analyzed in the cold room at a temperature of 5-10°C while for the remaining rat livers (Experiments IV, III and I), the different steps that had to be done in the cold were carried out in a constant temperature

water-glycerol bath of 0°C. The centrifuge shields were chilled prior to use. Centrifuging was done in a shorter time than when livers were analyzed in the cold room to prevent undue rise in temperature in the centrifuge tubes.

RNA was determined by measuring the UV absorption at 310, 280 and 260 μ[1]. Possible protein contamination was checked by determining the 260-310/280-310 ratio. A sample with a ratio below 1.34 was considered to be contaminated (Munro and Fleck, 1966). Such contamination was not found. According to Munro and Fleck (1966), 32 micrograms of rat liver RNA/ml is equivalent to an extinction of 1.000.

The method described by Ceriotti (1952) was used in the measurement of DNA. Two ml of the DNA solution to be tested was mixed with one ml of concentrated HCl and one ml of indole reagent in a screw capped culture tube. The solution was well shaken and placed in a boiling water bath for ten minutes. The tube was cooled in cold water and the pink color was removed by extracting thrice with 4 ml chloroform, centrifuging each time to give a completely clear yellow water phase. The optical density of the water phase was read at 490 μ[1]. Readings of the blanks under identical conditions were subtracted

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[1]Beckman Model DU Spectrophotometer.

from the sample readings. A standard curve[1] was also run under identical conditions.

Statistical Evaluation

Analysis of variance was carried out to determine differences among groups (Snedecor, 1961). The Duncan multiple-range test was used for testing differences between group means whenever the analysis of variance showed a significant F test $P < 0.05$ (Duncan, 1955; Harter, 1960). Correlation coefficients were used to relate the response of two parameters to dietary alterations. Group means are reported with standard errors of the mean.

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[1]Salmon Sperm DNA.

RESULTS

This study was done in four series: Experiments I, II, III and IV. The first experiment was designed to determine how protein depletion and immunization affect rats fed a stock ration. In Experiment I, group Sr was depleted for four weeks and repleted for two weeks, group Scly was given the stock ration continuously during the six weeks experimental period and group Scol was taken from the stock colony six days prior to autopsy. Group DD received the depletion diet for four weeks. All of the groups were injected with sheep erythrocytes (Srbc) as antigen (Table 1).

The second experiment was carried out to determine the immune response of rats to Salmonella O antigen and was preliminary in nature. Since several deaths occurred in this experiment a third experiment was conducted using a non-toxic antigen, Srbc. Diets in Experiments II and III were similar (Table 2). Diets R6 and C6 contained 6% protein from rice and corn respectively. Three other diets contained 10% protein from rice and fish (RF10), corn and fish (CF10) or a mixture of rice, corn and egg (RCE10). Protein in rice and corn is low; hence, there were no diets containing 10% protein from rice or corn alone.

Rice and corn were supplemented with animal sources of protein, fish and egg, in Experiments II and III, whereas in Experiment IV, rice was supplemented with another vegetable

source, mung beans, or with isolated amino acids. Rice was chosen because it is used more widely than corn as a staple food in some developing countries. Since fish is eaten in developing countries while egg is often used in developed countries, it was hoped that CF10 and RF10 would be comparable to RCE10.

Animals in Experiment II and III were depleted for three weeks and repleted for three and two weeks respectively. Repleted rats in Experiments I and IV were given a protein-free diet for four weeks and refed for two weeks. Animals in Experiments I, II and III were taken from our laboratory colony, whereas rats in Experiment IV were obtained from a commercial source. All rats, regardless of origin, were raised on stock diet in our laboratory until placed on the experimental diets.

Body Weight, Food Intake and Nitrogen Efficiency Ratio

Initial weights and ages of rats within an experiment were similar; however, rats in Experiments II and III were initially lighter than those in Experiments I and IV. All of the animals in this study lost approximately 1/5 of their body weight during the depletion period. Weight loss during the first week of depletion was approximately twice that in each of the succeeding weeks (Table 8). The rate of weight loss, however, did not seem to be related to initial weight or age. Relatively large variations in weight loss among groups in Experiment IV might have been due to inherent differences in the animals

Table 8. Mean initial weight loss per week and mean initial age in months

Experiment	Group	Mean Initial age	Initial weight	Weight loss on depletion	Mean weight loss 1st week	2nd week	3rd week	4th week
		months	gm	gm	gm	gm	gm	gm
I	Sr	4.4	502 ± 3	99 ± 6	36	25	19	19
	DD	4.2	500 ± 2	99 ± 5	42	26	15	15
II	R6	3.3	425 ± 4	75 ± 3	35	21	20	
	C6	3.4	428 ± 4	79 ± 3	40	22	17	
	RF10	3.2	429 ± 5	81 ± 3	40	24	17	
	CF10	3.2	422 ± 3	77 ± 5	40	22	15	
	RCE10	3.1	430 ± 5	78 ± 7	43	24	12	
IIIa	C6	3.7	431 ± 6	74 ± 5	39	21	15	
	CF10	3.6	430 ± 5	75 ± 3	36	21	18	
	RCE10	3.6	431 ± 5	77 ± 3	34	24	18	
IIIb	R6	4.1	430 ± 4	75 ± 3	39	22	14	
	RF10	3.9	437 ± 2	80 ± 4	43	21	16	
	Sr	3.8	439 ± 4	76 ± 4	39	23	15	
IV	R5	3.4	492 ± 4	109 ± 4	48	29	19	13
	RM5	3.4	496 ± 4	103 ± 6	48	27	17	11
	RAA6½	3.4	497 ± 5	97 ± 5	41	25	18	14
	RM7½	3.4	495 ± 4	93 ± 3	40	23	16	14
	RAA7½	3.4	492 ± 3	102 ± 4	46	27	17	12
	DD	3.3	484 ± 2	118 ± 8	51	35	19	14

themselves and to variation in food intake during depletion.

Upon repletion with the stock diet for 2 weeks, animals in Sr groups of Experiments I and III, regained their lost weight (Table 9). In the first experiment, although group Sr regained approximately 100 gm, the animals did not weigh as much as group Scly which had been fed a stock diet continuously throughout the six weeks experimental period and had gained 73 gm during this time. On the other hand, group Sr in Experiment III, which had also gained about 100 gm during refeeding had lost only 76 gm during the depletion period. Consequently, the autopsy weight exceeded initial weight by 29 gm. The discrepancy between groups Sr of Experiments I and III may be accounted for by the fact that the depletion period was longer in the first than in the third experiment.

In Experiment II, upon repletion for 3 weeks, rats on the corn diet gained only 21 gm whereas group R6 recovered 72 gm of the lost weight. Groups fed 10% protein diets regained their lost body weights. When corn was supplemented with fish (CF10), protein in the diet was increased from 6 to 10% and gain in weight increased three-fold. Supplementing rice with fish (RF10) to the same protein level resulted in weight gains $1\frac{1}{2}$ times those on the unsupplemented rice diet. Mean weight gains of groups RF10, CF10 and RCE10 were not significantly different in Experiment II. When similar groups were repleted for a shorter period (14 days, Experiment III), only rats fed RF10 and RCE10 recovered their body weight losses with refeeding

Table 9. Mean initial body weight, total weight loss on depletion, weight gain on repletion, food intake per day, total nitrogen intake and nitrogen efficiency ratio of diets - Experiments I, II and III

Experiment	Group	Mean initial body weight	Weight loss on depletion	Weight gain on repletion	Food intake per day	Total nitrogen intake	Nitrogen efficiency ratio[a]
		gm	gm	gm	gm	gm	
I	Sr[b]	502 ± 3[c]	99 ± 6	100 ± 6[e]	23.0 ± 0.8[d]		
	Scly	506 ± 3		73 ± 7[e]	23.3 ± 0.5[f]		
	Scol	488 ± 4					
	DD[b]	500 ± 2	99 ± 5		15.2 ± 0.5		
II[g]	C6	428 ± 4	79 ± 3	21 ± 5	19.5 ± 0.6	4.07 ± 0.12	4.9 ± 1.20
	CF10	422 ± 3	77 ± 5	93 ± 6	22.9 ± 0.6	8.20 ± 0.24	11.2 ± 0.51
	RCE10	430 ± 5	78 ± 7	118 ±18	23.9 ± 1.3	8.57 ± 0.48	13.4 ± 1.29
	R6	425 ± 4	75 ± 3	72 ±10	23.8 ± 1.4	5.03 ± 0.30	13.9 ± 1.24
	RF10	429 ± 5	81 ± 3	119 ± 6	23.9 ± 0.8	8.51 ± 0.30	13.9 ± 0.33
	Sc	383 ± 6					
	Sd	378 ± 5					

[a]Gain in weight/gm nitrogen intake.

[b]Depleted 28 days; repleted 14 days.

[c]Mean ± S.E.

[d]Vitamin supplements 5 or 6 weeks, see Table 1.

[e]Weight gain for six weeks.

[f]Food supplements, see Table 1.

[g]Depleted 21 days; repleted 22 days except Sc and Sd.

Table 9 (Continued)

Experiment	Group	Mean initial body weight gm	Weight loss on depletion gm	Weight gain on repletion gm	Food intake per day gm	Total nitrogen intake gm	Nitrogen efficiency ratio[a]
IIIa[e]	C6	431 ± 6	74 ± 5	28 ± 4	23.0 ± 1.2	2.99 ± 0.15	9.0 ± 0.95
	CF10	430 ± 5	75 ± 3	72 ± 4	24.1 ± 0.5	5.18 ± 0.11	13.9 ± 0.69
	RCE10	431 ± 5	77 ± 3	93 ± 4	25.0 ± 0.6	5.53 ± 0.13	16.7 ± 0.39
IIIb	R6	430 ± 4	75 ± 3	56 ± 3	23.9 ± 0.4	3.35 ± 0.06	16.7 ± 0.76
	RF10	437 ± 2	80 ± 4	98 ± 5	25.5 ± 0.7	5.78 ± 0.16	16.9 ± 0.53
	Sr	439 ± 4	76 ± 4	105 ± 5	24.3 ± 0.6		

[e]Depleted 21 days; repleted 14 days.

This suggests that fish in combination with corn was less suitable for tissue regeneration than was diet RF10, since a longer period of feeding was required for complete recovery of weight loss.

Mean daily food intake of rats fed diet C6 (19.5 gm) was lower than that of any other group in Experiment II (Table 9). Food intake in Experiment III was approximately the same for all groups. The source of rice for both experiments was from the same lot, whereas corn came from two different lots. The quality of corn used in Experiment III could have been better than that used in Experiment II, hence increasing the food intake in Experiment III.

Nitrogen efficiency ratio (NER) for diet C6 was the lowest (4.9) and differed significantly from that of all other diets in Experiment II (Tables 9, 10, 11). When corn was supplemented with fish, NER doubled in the second experiment and increased from 9.0 to 13.9 in the third experiment. The degree of improvement of the corn diet by the addition of fish cannot be evaluated with certainty. In both Experiments II and III the value for NER was lower for diet CF10 by 20-25% as compared to diet RF10, but the difference was statistically significant only in Experiment III (Tables 9, 10, 11). Unlike corn and fish-supplemented corn diets, the nitrogen efficiency ratio of rice was already high and was not increased further by the addition of fish or by the combination with corn plus egg.

Table 10. F values for analyses of variance for Table 9

Measurement	Experiment II		Experiment III	
	Degrees of freedom for treatments	F	Degrees of freedom for treatments	F
Weight gain on repletion gm	4	20.50**	5	48.27**
Food intake/day gm/day	4	3.89**	5	1.59
Nitrogen efficiency ratio	4	17.32**	4	23.71**

**Significant at $P < 0.01$.

Table 11. Results of Duncan's test for Table 9[a]

	Experiment II				
Group	C6	R6	CF10	RCE10	RF10
Weight gain on repletion, gm	21	72	93	118	119
$P < 0.01$					
$P < 0.05$					
Group	C6	CF10	R6	RCE10	RF10
Food intake/day gm/day	19.49	22.87	23.81	23.87	23.88
$P < 0.01$					
$P < 0.05$					
Group	C6	CF10	RCE10	RF10	R6
Nitrogen efficiency ratio	4.78	11.25	13.41	13.86	13.93
$P < 0.01$					
$P < 0.05$					

	Experiment III					
Group	C6	R6	CF10	RCE10	RF10	Sr
Weight gain on repletion, gm	28	56	72	93	98	105
$P < 0.01$						
$P < 0.05$						
Group	C6		CF10	R6	RCE10	RF10
Nitrogen efficiency ratio	9.01		13.91	16.67	16.71	16.89
$P < 0.01$						
$P < 0.05$						

[a]Any means not underscored by the same line are significantly different.

There are discrepancies in absolute figures for NER for comparable groups in the two experiments, though results in one corroborate those in the other.

As mentioned earlier, animals in Experiment II were repleted for three weeks whereas those in Experiment III were refed for two weeks only. Generally, very rapid weight gains occurred during the first few days of repletion. Thus, the differences in NER between Experiment II and III might be attributed to the length of repletion, since the initial spurt in weight gain during recovery would affect a two-week period more than a longer time span. Unfortunately, the design for Experiment II did not provide for an accurate determination of nitrogen efficiency during the first two weeks of repletion; hence no direct comparison could be made between the two experiments. The difference in NER could also be related to the kind of antigen that was used. In Experiment II, rats had lost approximately 15 gm during the first day after the injection of Salmonella O antigen. If the value for mean weight loss for this one day is added to the total mean weight gain of each group, NER becomes similar for both experiments (Table 12). Also, the rate of gain prior to injection of groups R6, RF10, CF10, RCE10 in Experiment II was similar to comparable groups in Experiment III.

In Experiment IV, a trend towards an increase in mean weight gain from 51 to 66 gm occurred when 1/3 of the rice protein was substituted with mung bean protein (RM5), but the

Table 12. A comparison of hypothetical nitrogen efficiency ratio of diets in Experiment II with nitrogen efficiency ratios of Experiment III

	Mean weight loss after injection gm	Mean weight gain on repletion gm	Hypothetical weight change gm	NER Experiment II	NER Experiment III
R6	-10	72	82	16.3	16.7
C6	-16	21	37	9.1	9.0
RF10	-15	119	134	15.7	16.9
CF10	-17	93	110	13.4	13.9
RCE10	-16	118	134	15.6	16.7

difference was not significant (Table 13). A marked increase in weight was noted when the protein level was raised and when mung beans or isolated amino acids, representing those in mung beans, were used to supplement rice at the higher levels. Weight recovery amounted to 110, 103 and 97 gm for groups RAA6½, RM7½ and RAA7½ respectively, approximately twice those obtained with 5% protein coming from either rice or rice-mung. Slight differences in weight gains of groups fed protein in excess of 5% were insignificant.

Nitrogen efficiency ratios overlapped greatly, ranging from 19.1 to 27.9. Although nitrogen utilization seemed least efficient with animals given R5 (19.1), the efficiency ratio was not significantly different from that found for other diets with the exception of RAA6½. This group made the greatest recovery of lost weight relative to the nitrogen intake, and seemed more efficient than rats receiving a similar diet at a higher level of nitrogen intake (RAA7½) (Tables 13, 14, 15).

Only a partial evaluation of the nutritive value of diets with respect to NER can be made because of the variation in protein level. That RAA6½ gave a significantly higher nitrogen efficiency ratio than RAA7½ suggests that RAA7½ could have exceeded the level of protein that would give optimum NER. However, the ratios of rice to amino acids were not identical in the two diets; the values were 3:1 and 2:1 for RAA6½ and RAA7½ respectively (Table 2). Therefore, possibilities exist that the amino acid pattern of the 3:1 ratio was more suitable

Table 13. Mean initial body weight, total weight loss on depletion, weight gain on repletion, food intake per day, total nitrogen intake and nitrogen efficiency ratio of diets - Experiment IV[a]

Group	Mean initial body weight gm	Weight loss on depletion gm	Weight gain on repletion gm	Food intake per day gm	Total nitrogen intake gm	Nitrogen efficiency ratio
R5	492 ± 4[b]	109 ± 4	51 ± 7	22.1 ± 1.1	2.59 ± 0.11	19.1 ± 1.92
RM5	496 ± 4	103 ± 6	66 ± 6	23.4 ± 1.0	2.74 ± 0.12	23.6 ± 1.51
RAA6½	497 ± 5	97 ± 5	110 ± 8	25.2 ± 0.7	3.95 ± 0.10	27.9 ± 1.79
RM7½	495 ± 4	93 ± 3	103 ± 6	25.8 ± 0.9	4.50 ± 0.19	22.8 ± 0.94
RAA7½	492 ± 3	102 ± 4	97 ± 6	23.9 ± 0.6	4.32 ± 0.11	22.5 ± 1.20
Scol	486 ± 2					
DD	484 ± 2	118 ± 8				
Sni	472 ± 7					

[a]Depleted 28 days; repleted 14 days except groups Scol and Sni.

[b]Mean ± S. E.

Table 14. F values for analyses of variance for Table 13

Measurement	Experiment IV Degrees of freedom for treatments	F
Weight gain on repletion, gm	4	15.85**
Food intake/day, gm/day	4	2.86*
Nitrogen efficiency ratio	4	4.31**

**Significant at $P < 0.01$.

*Significant at $P < 0.05$.

Table 15. Results of Duncan's test for Table 13[a]

	Experiment IV				
Group	R5	RM5	RAA7½	RM7½	RAA6½
Weight gain on repletion, gm	51	66	97	103	110
$P < 0.01$	———		———		
$P < 0.05$	- - - - - -		- - - - - - - - - - - -		
Group	R5	RM5	RAA7½	RAA6½	RM7½
Food intake/day gm/day	22.1	23.4	23.9	25.2	25.8
$P < 0.01$	———	———	———		
$P < 0.05$	- - - - - - - - - - - -	- - - - - - - - - - - - - - - -			
Group	R5	RAA7½	RM7½	RM5	RAA6½
Nitrogen efficiency ratio	19.07	22.50	22.83	23.61	27.86
$P < 0.01$	———	———	———		
$P < 0.05$	- -				

[a]Any means not underscored by the same line are significantly different.

than that of the 2:1 ratio and that the levels of protein in diets RM5 and RM7½ were too low and too high, respectively, to give maximum nitrogen utilization since both had similar efficiency ratios.

On the basis of the various parameters discussed so far, rice protein had a higher nutritive value than corn protein. Fish supplementation improved corn protein relatively more than rice protein. The combination of three proteins, rice, corn and egg was just as good as fish supplementation of rice. There was no evidence that partial substitution of rice with mung beans offered a specific advantage nutritionally. However, the increase in the protein level of the rice diet with inclusion of mung beans was beneficial.

Liver

Experiment I

Listed in Table 16 are mean weights, percent nitrogen, total nitrogen, percent fat, fat-free weight and nucleic acids of livers of rats given a depletion or stock diet.

Data from animals maintained on the stock ration or carried through the depletion regimen with or without repletion on the stock diet will be presented first since selected groups subjected to one of these treatments served as controls in the various experiments.

Table 16. Mean hepatic weight, percentage nitrogen and lipid, fat-free weight, total nitrogen and nucleic acids in livers of animals fed a stock ration or a protein-free diet

Experiment	Group	Weight gm	Fat-free weight gm	Fat % wet weight	Nitrogen % wet weight	Total nitrogen gm
I	Sr	13.16 ± 0.43	12.48 ± 0.41	5.2 ± 0.2	3.66 ± 0.03	0.48 ± .01
	Scly	14.66 ± 0.46	13.91 ± 0.44	5.1 ± 0.2	3.70 ± 0.04	0.54 ± .01
	Scol	11.91 ± 0.32	11.38 ± 0.32	4.4 ± 0.2	3.70 ± 0.04	0.44 ± .01
	DD	9.95 ± 0.31	9.19 ± 0.26	7.5 ± 0.8	2.62 ± 0.07	0.26 ± .004
II	Sc	14.52 ± 0.51	13.94 ± 0.50	4.0 ± 0.1	3.34 ± 0.10	0.48 ± .02
	Sd	14.00 ± 0.59	13.41 ± 0.56	4.2 ± 0.2	3.23 ± 0.10	0.45 ± .02
IIIb	Sr	12.45 ± 0.30	11.86 ± 0.29	4.7 ± 0.2	3.43 ± 0.06	0.43 ± .01
IV	Scol	12.34 ± 0.38	11.65 ± 0.36	5.6 ± 0.2	3.75 ± 0.05	0.46 ± .01
	Sni	12.13 ± 0.39	11.50 ± 0.37	5.2 ± 0.2	3.54 ± 0.06	0.43 ± .01
	DD	9.70 ± 0.35	8.79 ± 0.26	9.2 ± 0.9	2.42 ± 0.06	0.23 ± .004

Table 16 (Continued)

Experiment	Group	Total RNA (mg)	mg RNA / gm fat-free tissue	Total DNA (mg)	mg DNA / gm fat-free liver	RNA / DNA
I	Sr	97.0 ± 4.5	7.55 ± 0.35	37.1 ± 0.9	2.96 ± 0.11	2.62 ± 0.11
	Scly	101.0 ± 5.7	7.38 ± 0.26	38.2 ± 1.9	2.79 ± 0.10	2.66 ± 0.12
	Scol	90.5 ± 3.8	7.95 ± 0.29	33.7 ± 1.4	2.96 ± 0.10	2.70 ± 0.12
	DD					
II	Sc					
	Sd					
IIIb	Sr					
IV	Scol	100.0 ± 1.1	8.42 ± 0.19	28.6 ± 1.9	2.37 ± 0.13	3.60 ± 0.22
	Sni	93.2 ± 3.8	8.16 ± 0.27	26.6 ± 2.2	2.30 ± 0.15	3.65 ± 0.21
	DD	65.0 ± 1.8	7.41 ± 0.15	25.6 ± 1.7	2.94 ± 0.23	2.65 ± 0.19

Mean liver weights, fat-free liver weights and hepatic nitrogen were low in depleted animals and high in rats fed a stock ration (Experiments I and IV). The inverse was observed with hepatic fat which was high in depleted rats (7.5 to 9.2%) and low in animals given the stock ration (4.4 to 5.6%). Animals depleted and repleted on stock ration were able to regain their initial liver weights and composition (Sr, Experiments I, IIIb). Hepatic nucleic acid content was similar for repleted and non-repleted stock-fed animals in all experiments where these determinations were made (Tables 16, 17, 18).

Experiments II and III

In both Experiments II and III, mean liver weights of animals on the corn diet were low. Hepatic weights of group C6 almost equaled those of rats fed a protein-free diet in other experiments. Mean hepatic weights of groups C6 (10.95 gm) and CF10 (12.67) did not differ from each other in Experiment II but were significantly different in Experiment III. All the other groups except C6 had similar hepatic weights. Since there was fatty infiltration, absolute hepatic weight was of little value as a criterion for interpreting nutritive value.

In Experiment II fish-supplemented rice or corn or a mixture of rice, corn and egg gave almost identical concentrations of hepatic nitrogen averaging 2.94 to 2.95% (Tables 19, 20, 21). At the 6% level of protein intake, groups fed rice

Table 17. F values for analyses of variance for Table 16

Experiment I		
Measurement	Degrees of freedom for treatments	F
Liver weight, gm	3	26.60**
Hepatic N_2, %	3	118.26**
Hepatic fat, %	3	10.00**
Total RNA, mg	2	1.29
Total DNA, mg	2	2.54
RNA/DNA, mg/mg	2	0.10
RNA/gm fat-free liver, mg	2	0.93
DNA/gm fat-free liver, mg	2	0.87
Total nitrogen, gm/liver	3	92.60**

**Significant at $P < 0.01$.

or corn alone had significantly less hepatic nitrogen (2.46 and 2.62% respectively) than rats given the 10% supplemented diets. Likewise, total hepatic nitrogen was low for rats fed 6% and high for groups receiving 10% protein. These results were corroborated in Experiment III with one exception: concentration of hepatic nitrogen of group C6 exceeded that of group R6 significantly, and was similar to that of groups receiving the 10% protein diets. However, total nitrogen values were similar for groups C6 and R6. Total hepatic nitrogen of

Table 18. Results of Duncan's test for Table 16[a]

Experiment I

Group	DD	Scol	Sr	Scly
Liver weight, gm	9.95	11.91	13.16	14.66
$P < 0.01$		———		
$P < 0.05$				

Group	DD	Sr	Scol	Scly
Hepatic N_2, %	2.62	3.66	3.70	3.70
$P < 0.01$		———		
$P < 0.05$		- - -		

Group	Scol	Scly	Sr	DD
Hepatic fat, %	4.4	5.1	5.2	7.5
$P < 0.01$	———			
$P < 0.05$	- - -			

Group	DD	Scol	Sr	Scly
Total nitrogen, gm	0.26	0.44	0.48	0.54
$P < 0.01$		———		
$P < 0.05$				

[a]Any means not underscored by the same line are significantly different.

Table 19. Mean hepatic weight, percentage nitrogen and lipid, fat-free weight, total nitrogen in livers - Experiments II and III

Experiment	Group	Weight, gm	Nitrogen		Lipid	Fat-free
			% wet weight	gm/liver	% wet weight	weight, gm
II	C6	10.95 ± 0.39	2.62 ± 0.06	0.29 ± 0.01	7.0 ± 0.9	10.17 ± 0.33
	CF10	12.67 ± 0.45	2.94 ± 0.15	0.42 ± 0.01	5.2 ± 0.1	12.07 ± 0.43
	RCE10	14.52 ± 0.64	2.95 ± 0.12	0.43 ± 0.03	4.7 ± 0.4	13.83 ± 0.59
	R6	13.03 ± 0.64	2.46 ± 0.08	0.32 ± 0.01	9.6 ± 0.9	11.75 ± 0.50
	RF10	13.71 ± 0.45	2.94 ± 0.04	0.40 ± 0.01	5.4 ± 0.2	12.83 ± 0.42
	Sc	14.52 ± 0.51	3.34 ± 0.10	0.48 ± 0.02	4.0 ± 0.1	13.94 ± 0.50
	Sd	14.00 ± 0.59	3.23 ± 0.10	0.45 ± 0.02	4.2 ± 0.2	13.41 ± 0.56
IIIa	C6	9.56 ± 0.29	3.07 ± 0.13	0.27 ± 0.01	9.3 ± 1.1	8.90 ± 0.32
	CF10	11.61 ± 0.36	3.20 ± 0.02	0.37 ± 0.01	5.7 ± 0.3	10.94 ± 0.33
	RCE10	13.02 ± 0.59	3.09 ± 0.02	0.40 ± 0.01	5.3 ± 0.5	12.32 ± 0.53
IIIb	R6	13.02 ± 0.81	2.35 ± 0.11	0.30 ± 0.01	12.2 ± 1.6	11.35 ± 0.56
	RF10	13.00 ± 0.48	3.12 ± 0.07	0.40 ± 0.02	5.4 ± 0.2	12.30 ± 0.45
	Sr	12.45 ± 0.30	3.43 ± 0.06	0.43 ± 0.01	4.7 ± 0.2	11.86 ± 0.29

Table 20. F values for analyses of variance for Table 19

Measurement	Experiment II		Experiment III	
	Degrees of freedom for treatments	F	Degrees of freedom for treatments	F
Liver weight, gm	6	5.94**	5	7.19**
Hepatic nitrogen, %	4	8.51**	5	17.06**
Hepatic fat, %	4	10.39**	5	13.35**
Total nitrogen, gm	4	17.10**	5	27.80**

**Significant at $P < 0.01$.

Table 21. Results of Duncan's test for Table 19[a]

Experiment II

Group	R6	C6	RF10	CF10	RCE10	Sd	Sc
Hepatic nitrogen, %	2.46	2.62	2.94	2.94	2.95	3.23	3.34
P < 0.01	———		———			———	
P < 0.05	- - - - - -		- - - - - - - - - -			- - - - - -	

Group	Sc	Sd	RCE10	CF10	RF10	C6	R6
Hepatic fat, %	4.0	4.2	4.7	5.2	5.4	7.0	9.6
P < 0.01	———			———			
P < 0.05	- - - - - - - - - - - - - - - - - -						

Group	C6	CF10	R6	RF10	Sd	RCE10	Sc
Liver weight, gm	10.95	12.67	13.03	13.71	14.00	14.52	14.52
P < 0.01	———	———					
P < 0.05	- - - - - - -	- - - - - - - - - -	- - - - - - - - - - - - - - - - - -				

Group	C6	R6	RF10	CF10	RCE10	Sd	Sc
Total nitrogen, gm	.28	.32	.40	.43	.43	.45	.48
P < 0.01				———			
P < 0.05				- - - - - -			

[a]Any means not underscored by the same line are significantly different.

Table 21 (Continued)

Experiment III

Group	R6	C6	RCE10	RF10	CF10	Sr
Hepatic nitrogen, %	2.35	3.07	3.09	3.12	3.20	3.43
P < 0.01		———	———	———	———	———
P < 0.05		- - -	- - -	- - -		
		- - -	- - -	- - -	- - -	
					- - -	- - -

Group	Sr	RCE10	RF10	CF10	C6	R6
Hepatic fat, %	4.7	5.3	5.4	5.7	9.3	12.2
P < 0.01	———	———	———	———	———	———
P < 0.05	- - -	- - -	- - -	- - -		

Group	C6	CF10	Sr	RF10	R6	RCE10
Liver weight, gm	9.56	11.61	12.45	13.00	13.02	13.02
P < 0.01		———	———	———	———	———
P < 0.05		- - -	- - -	- - -	- - -	- - -

Group	C6	R6	CF10	RCE10	RF10	Sr
Total nitrogen, gm	0.27	0.30	0.37	.40	.41	.43
P < 0.01	———	———		———	———	———
			———	———	———	
P < 0.05	- - -	- - -		- - -	- - -	- - -
			- - -	- - -	- - -	

group CF10 was significantly lower ($P < 0.05$) than that of groups RF10 and RCE10.

Pale livers indicative of fatty infiltration were rarely seen in Experiments II and III except in rats fed rice alone as a source of protein. This subjective observation could be correlated in part with chemical data. Mean percentage hepatic fat was highest in animals fed diet R6 in both experiments. Hepatic lipid concentration for group R6 differed from those in group C6 in Experiment II and Experiment III ($P < 0.05$). In the latter experiment, hepatic fat concentrations were 12.2 and 9.3% for R6 and C6 respectively while corresponding values for Experiment II were 9.6 and 7.0% (Tables 19, 20, 21). In both experiments, feeding either diet R6 or C6 resulted in hepatic fat concentrations almost twice that of those fed the stock diet, while supplementation reduced the values to the level of rats given the stock ration. The degree of fat infiltration in livers of groups C6 and R6 was greater in Experiment III than in Experiment II.

Hepatic RNA and DNA were not determined in the second experiment. In Experiment III these cellular constituents were expressed as amount per whole liver or in various ratios (Tables 22, 23, 24). Since livers of rats from groups R6 and RF10 were homogenized a year before they were analyzed for nucleic acids whereas livers from groups C6, CF10 and RCE10 were freshly homogenized from the frozen state there is a possibility that the difference in methods might have affected

Table 22. Hepatic RNA and DNA of animals in Experiment III

Experiment	Group	Fat-free weight gms	Total RNA mg	gm RNA / gm fat-free liver	Protein/RNA mg/mg
IIIa	C6	8.98 ± 0.32	70.1 ± 2.2	7.90 ± 0.37	26.4 ± 1.9
	CF10	10.94 ± 0.33	83.8 ± 1.7	7.70 ± 0.20	27.6 ± 0.6
	RCE10	12.32 ± 0.53	89.6 ± 5.9	7.25 ± 0.27	28.7 ± 1.7
IIIb	R6	11.35 ± 0.56	80.0 ± 1.8	7.16 ± 0.29	24.1 ± 0.7
	RF10	12.30 ± 0.45	95.3 ± 4.8	7.74 ± 0.26	26.9 ± 1.0

Table 22 (Continued)

Experiment	Group	Total DNA mg	mg DNA / gm fat-free liver	Protein/DNA mg/mg	RNA/DNA
IIIa	C6	32.2 ± 1.6	3.61 ± 0.17	57.5 ± 3.5	2.20 ± 0.12
	CF10	32.0 ± 1.1	2.96 ± 0.17	73.4 ± 3.7	2.65 ± 0.11
	RCE10	35.4 ± 1.8	2.90 ± 0.14	72.4 ± 4.0	2.59 ± 0.02
IIIb	R6	26.2 ± 0.9	2.33 ± 0.07	73.1 ± 2.7	3.08 ± 0.09
	RF10	28.1 ± 0.9	2.30 ± 0.06	90.1 ± 2.2	3.40 ± 0.11

Table 23. F values for analyses of variance for Table 22

Experiment III		
Measurement	Degrees of freedom for treatments	F
Total RNA, mg	4	6.66**
Total DNA, mg	4	7.49**
RNA/DNA, mg/mg	4	11.59**
RNA/gm fat-free liver, mg	4	1.30
DNA/gm fat-free liver, mg	4	16.40**
Protein/RNA, mg/mg	4	1.84
Protein/DNA, mg/mg	4	12.17**

**Significant at $P < 0.01$.

the data. Since the effect cannot be determined, it will be assumed in subsequent discussions that observations were consequences of dietary treatment.

Total hepatic RNA was relatively low with the two unsupplemented diets fed at the 6% protein level while the two supplemented rice-containing diets gave significantly higher amounts. Diet CF10 had an intermediate position. However, the range of all means was relatively narrow, from 70.1 to 95.3 mg. No significant differences existed in mg RNA/gm fat-free liver.

Table 24. Results of Duncan's test for Table 22[a]

	Experiment III				
Group	C6	R6	CF10	RCE10	RF10
Total RNA, mg	70.1	80.0	83.8	89.6	95.3
P < 0.01		———	———	———	
P < 0.05	- - - - - - -	- - - - - - -	- - - - - - -	- - - - - - -	
Group	R6	RF10	CF10	C6	RCE10
Total DNA, mg	26.2	28.1	32.0	32.2	35.4
P < 0.01	———	———	———	———	
P < 0.05	- - - - - - -	- - - - - - -	- - - - - - -	- - - - - - -	
Group	C6	RCE10	CF10	R6	RF10
RNA/DNA, mg/mg	2.20	2.59	2.65	3.08	3.40
P < 0.01	———	———	———	———	
P < 0.05		- - - - - - -	- - - - - - -	- - - - - - -	
Group	RF10	R6	RCE10	CF10	C6
DNA/gm fat-free liver	2.30	2.33	2.90	2.96	3.61
P < 0.01	———	———	———		
P < 0.05	- - - - - - -	- - - - - - -	- - - - - - -		
Group	C6	RCE10	R6	CF10	RF10
Protein/DNA, mg/mg	57.5	72.4	73.1	73.4	90.1
P < 0.01		———	———	———	
P < 0.05		- - - - - - -	- - - - - - -	- - - - - - -	

[a]Any means not underscored by the same line are significantly different.

Values for total DNA were somewhat different. Livers of rats fed diet C6 had relatively low amounts of RNA but ranked high with respect to total DNA. The reverse was true for group RF10.

Total DNA in groups C6 and CF10 exceeded amounts found with groups R6 and RF10. The value was higher in groups C6 (32.2 mg) than in group R6 (26.2 mg). Likewise, it was higher in CF10 (32.0 mg) than in RF10 (28.1 mg) ($P < 0.05$).

DNA concentration in fat-free tissue followed a pattern similar to that of total DNA. Values for DNA per gram of fat-free tissue decreased when protein was increased from 6 to 10% by the addition of fish to corn, or when rats were given the RCE mixture, but not when rice was supplemented with fish. Although total DNA content did not differ between groups C6 and CF10, there was a difference of approximately two grams in fat-free liver weights between the two groups; hence, the concentration of DNA changed when expressed on the fat-free basis. In contrast, there were no differences in fat-free liver weights or total DNA content between groups R6 and RF10; consequently, DNA concentration in fat-free tissue remained constant. Thus, supplementing corn with fish altered the amounts of DNA per gram of fat-free tissue but similar supplementation of rice did not affect that ratio.

The nucleic acid content of hepatic tissue is frequently evaluated in terms of the RNA/DNA ratio. This parameter has also been used to assess the nutritive value of proteins.

The RNA/DNA ratio was low in C6-fed rats. A significant increase occurred when corn was supplemented, but not when rice was supplemented with fish. It is possible that because of the relatively high efficiency ratio of the rice protein, fish supplementation did not affect or alter RNA/DNA ratio as it did when added to corn. Hepatic RNA/DNA ratio of rats fed RCE10 was similar to CF10 but was significantly lower than that of group RF10 ($P < 0.05$) (Tables 22, 23, 24).

Experiment IV

In Experiment IV, concentration of hepatic nitrogen was highest in groups given the stock diets with values of 3.54 and 3.75% for groups Sni and Scol respectively (Table 25). After depletion, the value had dropped to 2.42%. As was expected, among the experimental diets, hepatic nitrogen tended to increase while hepatic fat decreased with increasing amounts of protein and substitution or supplementation. Total hepatic nitrogen was lower in the depleted animals than in groups R5 and RM5. Groups RAA6½, RM7½ and RAA7½ had similar mean total hepatic nitrogen (Table 25).

Most of the livers of rats on the experimental diets in the fourth study were judged to be slightly pale (RAA7½) or very pale (R5), and five out of 60 had a pus-like sac. There were fewer pale livers noted in rats fed the depletion diet than in those refed with diet R5. This observation is confirmed by chemical data. The concentration of fat in group R5

Table 25. Mean weight, percentage nitrogen and lipid, and total nitrogen in liver of animals in Experiment IV

Group	Weight gm	Nitrogen % wet weight	Nitrogen gm	Lipid % wet weight
R5	11.73 ± 0.93	2.64 ± 0.14	0.30 ± 0.01	14.6 ± 2.0
RM5	12.07 ± 0.46	2.70 ± 0.05	0.32 ± 0.01	11.2 ± 1.0
RAA6½	14.20 ± 0.61	2.74 ± 0.07	0.38 ± 0.01	11.2 ± 1.4
RM7½	12.98 ± 0.61	2.86 ± 0.09	0.36 ± 0.01	11.3 ± 1.1
RAA7½	12.33 ± 0.39	3.03 ± 0.07	0.37 ± 0.01	7.8 ± 0.6
Scol	12.34 ± 0.38	3.75 ± 0.05	0.46 ± 0.01	5.6 ± 0.2
DD	9.70 ± 0.35	2.42 ± 0.06	0.23 ± 0.00	9.2 ± 0.9
Sni	12.13 ± 0.39	3.54 ± 0.05	0.43 ± 0.01	5.2 ± 0.2

was 14.6% while in the depleted rats it was 9.2% ($P < 0.05$) (Tables 26 and 27).

In general, there was an inverse relationship between percent hepatic nitrogen and percent hepatic fat in all groups ($r = -.792$)[1]. This relationship was clear cut for groups R5 and RAA7½. Group R5 had the highest hepatic fat and the lowest mean nitrogen value while the opposite was true of RAA7½.

[1] $P < 0.05$.

Table 26. F values for analyses of variance for Table 25

Measurement	Experiment IV Degrees of freedom for treatments	F
Liver weight, gm	7	4.80**
Hepatic nitrogen, %	7	34.61**
Hepatic fat, %	7	8.90**
Total nitrogen, gm	7	42.21**

**Significant at $P < 0.01$.

Mean total RNA for the depleted rats was the lowest (65.5 mg) while rats on the stock ration (Scol) had the highest value (100.0 mg). However, only groups fed the experimental diets at the 5% protein level differed from group Scol (Tables 28, 29, 30).

Values for total DNA ranged from 25.6 to 33.5 mg. An analysis of variance failed to show a difference among group means. However, the group on stock ration gave the highest value, the depleted group was the lowest and the rest of the groups were intermediate. DNA concentration was fairly constant despite changes in kind and amount of protein in the repletion diets.

Table 27. Results of Duncan's test for Table 25[a]

Experiment IV

Group	DD	R5	RM5	RAA6½	RM7½	RAA7½	Sni	Scol
Hepatic nitrogen, %	2.42	2.64	2.70	2.74	2.86	3.03	3.54	3.75
P < 0.01								
P < 0.05								

Group	Sni	Scol	RAA7½	DD	RM5	RAA6½	RM7½	R5
Hepatic fat, %	5.23	5.60	7.76	9.23	11.20	11.21	11.29	14.60
P < 0.01								
P < 0.05								

Group	DD	R5	RM5	Sni	RAA7½	Scol	RM7½	RAA6½
Liver weight, gm	9.70	11.73	12.07	12.13	12.33	12.34	12.98	14.20
P < 0.01								
P < 0.05								

Group	DD	R5	RM5	RM7½	RAA7½	RAA6½	Sni	Scol
Total nitrogen, gm	.23	.30	.32	.36	.37	.38	.43	.46
P < 0.01								
P < 0.05								

[a]Any means not underscored by the same line are significantly different.

Table 28. Hepatic RNA and DNA of animals in Experiment IV

Group	gm fat-free liver weight	Total RNA mg/liver	mg RNA/gm fat-free liver	Protein/RNA mg/mg
R5	9.87 ± 0.61	80.8 ± 2.8	8.42 ± 0.41	23.2 ± 0.5
RM5	10.70 ± 0.38	85.7 ± 3.0	8.05 ± 0.26	23.7 ± 0.6
RAA6½	12.53 ± 0.40	101.3 ± 3.5	8.12 ± 0.20	23.7 ± 0.4
RM7½	11.48 ± 0.49	97.9 ± 3.6	8.54 ± 0.11	23.7 ± 0.6
RAA7½	11.38 ± 0.36	90.9 ± 3.7	8.09 ± 0.12	25.6 ± 0.7
Scol	11.65 ± 0.36	100.0 ± 1.1	8.42 ± 0.19	29.3 ± 0.6
DD	8.79 ± 0.26	65.0 ± 1.8	7.41 ± 0.15	22.6 ± 0.4
Sni	11.50 ± 0.37	93.2 ± 3.8	8.16 ± 0.27	29.1 ± 1.0

Group	Total DNA gms/liver	mg DNA / gm fat-free liver	Protein/DNA mg/mg	RNA/DNA
R5	29.6 ± 1.7	3.18 ± 0.32	65.4 ± 4.3	2.83 ± 0.18
RM5	30.6 ± 2.2	2.92 ± 0.26	70.3 ± 5.6	2.92 ± 0.20
RAA6½	33.5 ± 2.0	2.70 ± 0.17	74.5 ± 3.9	3.07 ± 0.20
RM7½	31.6 ± 1.6	2.83 ± 0.21	74.3 ± 3.9	3.20 ± 0.21
RAA7½	29.6 ± 1.8	2.65 ± 0.20	82.7 ± 6.1	3.19 ± 0.23
Scol	28.6 ± 1.9	2.37 ± 0.13	105.5 ± 6.4	3.60 ± 0.22
DD	25.6 ± 1.7	2.94 ± 0.23	59.7 ± 4.0	2.65 ± 0.19
Sni	26.6 ± 2.2	2.30 ± 0.15	105.6 ± 6.0	3.65 ± 0.21

Table 29. F values for analyses of variance for Table 28

Measurement	Experiment IV Degrees of freedom for treatments	F
Total RNA, mg	7	12.40**
Total DNA, mg	7	1.78
RNA/DNA, mg/mg	7	2.69*
RNA/gm fat-free liver, mg/gm	7	1.93
DNA/gm fat-free liver, mg/gm	7	1.69
Protein/RNA, mg/mg	7	15.83**
Protein/DNA, mg/mg	7	10.77**

**Significant at $P < 0.01$.

*Significant at $P < 0.05$.

Since there was no difference among group means by analysis of variance with respect to total DNA, RNA/DNA ratio gave results similar to total RNA alone. Again RNA/DNA ratio was low in the depleted group (2.65) and high in stock-fed rats (3.60 and 3.65) with intermediate values for rats given the experimental diets ranging from 2.83 to 3.20. Ratios in groups fed the 5% protein diets were significantly lower than those fed the stock ration.

When a comparison between immunized, undepleted stock rats (Scol) and non-immune, stock-fed rats (Sni) was made,

Table 30. Results of Duncan's test for Table 28[a]

Experiment IV

Group	DD	R5	RM5	RAA7½	RAA6½	Sni	RM7½	Scol
Total RNA, mg	65.5	80.8	85.7	90.9	91.6	93.2	97.9	100.0
P < 0.01								
P < 0.05								

Group	DD	R5	RM5	RAA6½	RAA7½	RM7½	Scol	Sni
RNA/DNA, mg/mg	2.65	2.83	2.92	3.09	3.19	3.20	3.60	3.65
P < 0.01								
P < 0.05								

Group	DD	R5	RM5	RM7½	RAA6½	RAA7½	Scol	Sni
Protein/DNA, mg/mg	59.7	65.4	70.3	74.3	74.5	82.7	105.5	105.6
P < 0.01								
P < 0.05								

Group	DD	R5	RM7½	RM5	RAA6½	RAA7½	Sni	Scol
Protein/RNA, mg/mg	22.6	23.2	23.7	23.7	23.7	25.6	29.1	29.3
P < 0.01								
P < 0.05								

[a]Any means not underscored by the same line are significantly different.

(Experiment IV) immunization did not alter the nucleic acid contents of the liver. Likewise, hepatic nitrogen and lipid were not affected by immunization.

Spleen and Immune Response

In general, spleen weights were markedly decreased in depletion but upon repletion, spleen weights increased with body weight (Tables 31, 32a, 32b).

Experiment I

In Experiment I, spleen weight, total and nucleated cell counts, antibody-forming cells, agglutinins and hemolysins did not differ between repleted and non-repleted stock-fed animals. Although spleen weights of depleted rats were low, antibody titers did not differ significantly from those of groups fed the stock ration (Tables 31 and 32).

Experiment II

Mean spleen weight of rats fed corn alone as the protein source was significantly lower than that of rats fed either a 10% protein diet or the stock ration. Mean spleen weight of group R6 did not differ from any of the other groups (Table 33). Hence, fish supplementation affected spleen weights of C6-fed rats but did not change spleen weights of R6-fed rats appreciably.

Table 31. Mean spleen weights, cell counts, antibody-forming cells and agglutinins and hemolysins of animals fed a stock ration or a protein-free diet

Experiment	Group	Spleen weight gm	Cell count / spleen x 10^7	Nucleated cells / spleen %	Nucleated cell count x 10^7
I	Sr	0.88 ± 0.04	170 ± 16	35.6 ± 2.7	60 ± 6
	Scly	0.99 ± 0.01	174 ± 10	35.4 ± 1.5	62 ± 4
	Scol	0.93 ± 0.04			
	DD	0.64 ± 0.04			

Experiment	Group	Plaque count / spleen log	Agglutinins log	Hemolysins log
I	Sr	1.96 ± 0.18	1.78 ± 0.15	3.23 ± 0.17
	Scly	2.12 ± 0.13	1.70 ± 0.18	3.01 ± 0.27
	Scol		1.85 ± 0.19	3.14 ± 0.33
	DD		1.75 ± 0.23	2.98 ± 0.24

Table 32a. F values for analyses of variance for Table 31

Measurement	Experiment I Degrees of freedom for treatments	F
Spleen weight, gm	1	3.72
Agglutinins, log	1	0.13
Hemolysins, log	1	0.50
Plaque count/spleen, log	1	0.52
Total spleen cell count x 10^7	1	0.04
Nucleated cells, %	1	0.009

Table 32b. Results of Duncan's test for Table 31[a]

Group	Experiment I DD	Scol	Sr	Scly
Spleen weight, gm	0.64	0.88	0.98	0.99
$P < 0.01$		———	———	———
$P < 0.05$		- - -	- - -	- - -

[a]Any means not underscored by the same line are significantly different.

Mean agglutinin titers in Experiment II were similar on a statistical basis. However, certain trends might be pointed out. Agglutinin titers on the 6th day after injection of Salmonella O antigen were highest in rats fed CF10 and lowest with the RCE10 mixture (Tables 33, 34 and Figure 1). By the 10th day after injection, mean agglutinin titers had decreased in all groups. The decrease seemed to be greatest in rats that had highest mean titer on the 6th day after injection (CF10). On the 14th day, there was a further decrease in agglutinin titers; however, average titers of groups RF10 and Sc tended to level off, whereas other groups showed a continuous decline in serum agglutinins. Titers of animals fed rice showed the largest drop; next in descending order were titers of groups Sd, CF10, C6, RCE10, Sc and RF10. Concentration of the antigen may be one reason for the difference in rate of decline in titers between Sc and Sd. The titers of groups C6 and CF10 dropped at about the same rate, whereas titers of group R6 showed greatest decrease while those of RF10 changed relatively little. The difference in the rates of decline in titers may be due in part to the quality of the diet, and also to the kind of antibody (19S vs 7S) that is dominant during the 6th and 14th day after immunization. Results suggest that where the concentration of the injected dose was the same, the quantity and quality of the diet affected the decline in antibody titers. This may be explained in part by a report of Guggenheim and Buechler (1948). These authors were able to demonstrate a

Table 33. Mean spleen weights and agglutinin titers on the 6th, 10th and 14th day after immunization - Experiment II

Group	Spleen weight gm	Agglutinin 6th day log	Agglutinin 10th day log	Agglutinin 14th day log
C6	0.66 ± .10	2.07 ± .14	1.82 ± .10	1.59 ± .14
CF10	0.89 ± .13	2.39 ± .16	2.03 ± .13	1.88 ± .13
RCE10	0.96 ± .06	1.83 ± .34	1.65 ± .24	1.39 ± .15
R6	0.76 ± .07	2.05 ± .10	1.78 ± .17	1.43 ± .12
RF10	0.94 ± .07	2.11 ± .17	1.91 ± .20	1.90 ± .17
Sc	0.94 ± .05	1.99 ± .25	1.77 ± .16	1.69 ± .13
Sd	0.87 ± .02	2.26 ± .14	2.02 ± .14	1.71 ± .14

Table 34a. F values for analyses of variance for Table 33

Measurement	Experiment II Degrees of freedom for treatments	F
Spleen weight, gm	6	2.59*
Agglutinins, log		
6th day	6	0.83
10th day	6	0.66
14th day	6	1.85

*Significant at $P < 0.05$.

Table 34b. Results of Duncan's test for Table 33[a]

	Experiment III						
Group	C6	R6	Sd	CF10	RF10	Sc	RCE10
Spleen weight	.66	.76	.87	.89	.94	.94	.96
$P < 0.01$							
$P < 0.05$							

[a]Any means not underscored by the same line are significantly different.

a diminished bactericidal and phagocytic power of peritoneal fluid of rats fed low protein diets and proteins of low biological value. The rate of decrease in antibody titers may be significant in that the longer the rate of decline, the longer the immunity of the animal; hence, the better his resistance to infection. Regardless of the mechanisms involved antibody titers in rats fed such diets may persist as a result of inefficient removal of antigen from the circulation.

Experiment III

In Experiment III, mean spleen weights of rats fed rice or corn were lower than those of other groups (Tables 35, 36, 37). There was a tendency towards increases in plaque counts and in both antibodies when corn was supplemented, but this could not be verified statistically (Figure 2). However, there was a significant increase in plaque counts when rice

Figure 1. Mean agglutinin titers on the 6th, 10th and 14th day after injection of antigen - Experiment II

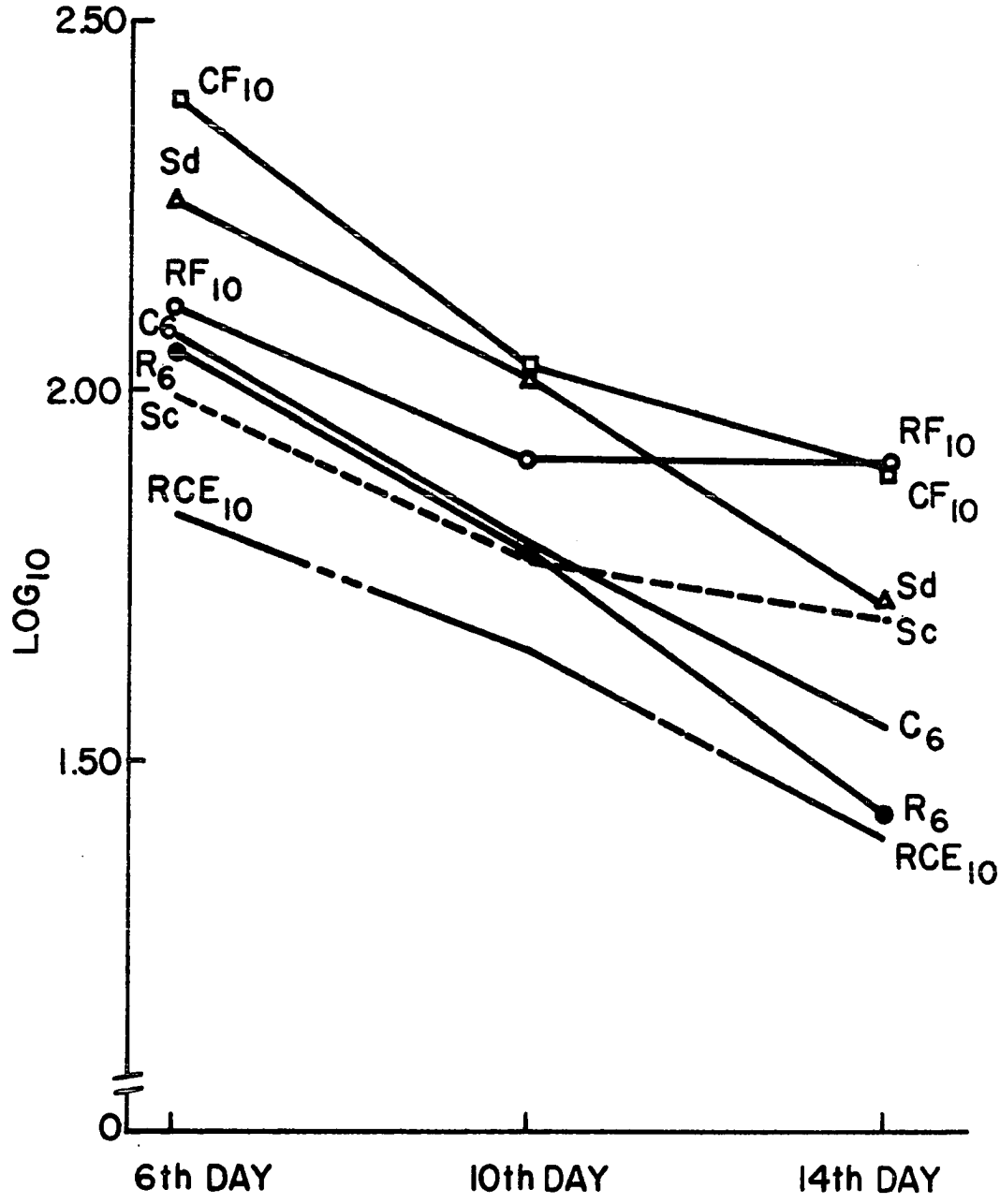

2.50
2.00
1.50
0
LOG10
CF10
Sd
RF10
C6
R6
Sc
RCE10
RF10
CF10
Sd
Sc
C6
R6
RCE10
6th DAY
10th DAY
14th DAY

Table 35. Mean spleen weights, antibody-forming cells, agglutinins and hemolysins - Experiment III

Experiment	Group	Spleen weight	Plaque count / spleen[a]	Agglutinin	Hemolysin	Agglutinin AFC	Hemolysin AFC
		gm	log	log	log		
IIIa	C6	0.55 ± .03	2.37 ± .11	0.96 ± .11	2.94 ± .25	.07	9.09
	CF10	0.71 ± .04	2.65 ± .14	1.07 ± .08	3.12 ± .14	.04	6.10
	RCE10	0.76 ± .01	2.74 ± .11	1.05 ± .02	2.96 ± .20	.03	5.21
IIIb	R6	0.59 ± .03	1.74 ± .24	0.94 ± .11	2.99 ± .16	.31	20.24
	RF10	0.76 ± .04	2.35 ± .18	1.19 ± .16	2.95 ± .19	.08	4.04
	Sr	0.77 ± .04	1.84 ± .34	0.99 ± .15	2.79 ± .18	.22	16.59

[a] 7 to 10 animals to a group.

Table 36. F values for analyses of variance for Table 35

Measurement	Experiment III Degrees of freedom for treatments	F
Spleen weight, gm	5	7.18**
Agglutinins, log	5	0.58
Hemolysins, log	5	0.31
Total spleen cell count x 10^7	5	1.71
Plaque count/spleen, log	5	4.48**

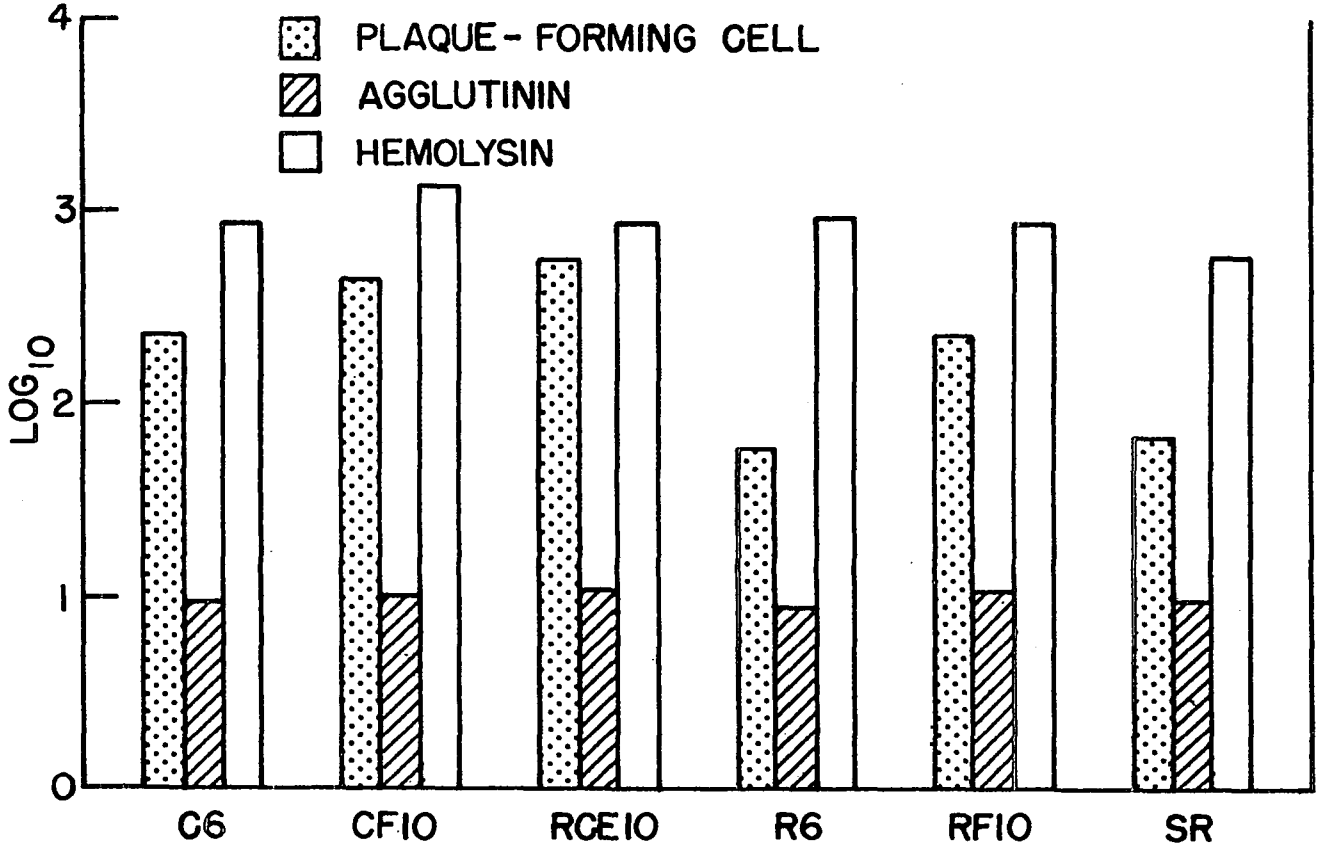
**Significant at $P < 0.01$.

Table 37. Results of Duncan's test for Table 35[a]

	Experiment III					
Group	C6	R6	CF10	RCE10	RF10	Sr
Spleen weight, gm	.55	.59	.71	.76	.76	.77
$P < 0.01$	———		———			
$P < 0.05$	- - - - - -		- - - - - - - - - - - - - -			
Group	R	S	RF	C	CF	RCE
Plaque count, log	1.74	1.84	2.35	2.37	2.65	2.74
$P < 0.01$	———		———			
$P < 0.05$	- - - - - -	- - - - - -	- - - - - - - - - - - - - -			

[a]Any means not underscored by the same line are significantly different.

Figure 2. Mean plaque counts, agglutinins and hemolysins - Experiment III


PLAQUE-FORMING CELL
AGGLUTININ
HEMOLYSIN
LOG10
4
3
2
1
0
C6
CF10
RCE10
R6
RF10
SR

was supplemented with fish or when the rice-corn-egg combination was compared with either one of the 6% protein diets. With supplementation of rice agglutinin titers appeared to increase but hemolysin titers did not; neither antibody was significantly affected.

When titers of hemolysin and agglutinin were related to plaque-forming cells, a decrease in titers per AFC was observed when the amount of protein was raised from 6 to 10% (Table 35). Correlation coefficients based on group means showed no relationship between AFC and agglutinin or hemolysin and between agglutinin and hemolysin (Table 38).

Experiment IV

Table 38 shows the progressive increase in spleen weights from 5% protein to $6\frac{1}{2}$ to $7\frac{1}{2}$ to stock diet in Experiment IV. Plaque counts per spleen paralleled spleen weight. As plaque counts increased, there was a parallel increase in agglutinin and hemolysin titers in groups fed the experimental and stock diets (Tables 38, 39, 40 and Figure 3). Although plaque count was lowest for the depleted rats, circulating antibodies did not always give the lowest value as measured by all these parameters.

Hemolysin and agglutinin titers per AFC were high in depleted and low in stock-fed animals. In rats fed the rice-mung bean combination, hemolysin and agglutinin titers per AFC appeared to increase with increase in nitrogen intake. Output

Table 38. Mean spleen weights, cell counts, antibody-forming cells, agglutinins and hemolysins - Experiment IV

Experiment	Group	Spleen weight	Cell count / spleen	Nucleated cells / spleen	Nucleated cell count / spleen
		gm	x 10^7	%	x 10^7
IV	R5	0.51 ± .03	52 ± 7	56.8 ± 5.0	29 ± 5
	RM5	0.57 ± .02	56 ± 6	56.9 ± 4.6	31 ± 12
	RAA6½	0.65 ± .03	76 ± 8	56.1 ± 3.5	43 ± 6
	RM7½	0.63 ± .02	54 ± 4	57.5 ± 3.0	31 ± 3
	RAA7½	0.64 ± .04	67 ± 7	53.9 ± 5.6	37 ± 6
	Scol	0.70 ± .05	146 ± 22	48.1 ± 4.0	66 ± 8
	DD	0.47 ± .03	56 ± 6	32.3 ± 3.2	17 ± 2
	Sni	0.66 ± .03	70 ± 8	53.0 ± 3.9	

Table 38 (Continued)

Experiment	Group	Agglutinin log	Hemolysin log	Plaque count spleen log	Hemolysin AFC	Agglutinin AFC
IV	R5	1.00 ± .17	1.48 ± .17	1.39 ± .15	1.00	0.54
	RM5	1.20 ± .16	1.55 ± .14	1.66 ± .23	1.90	0.67
	RAA6½	1.32 ± .21	1.73 ± .18	1.86 ± .16	1.20	0.57
	RM7½	1.60 ± .22	2.03 ± .21	1.99 ± .18	2.03	1.11
	RAA7½	1.76 ± .14	2.11 ± .15	2.17 ± .21	2.30	0.91
	Scol	1.54 ± .16	1.81 ± .12	2.26 ± .14	0.41	0.23
	DD	1.16 ± .09[a]	1.68 ± .13[a]	1.39 ± .14	2.55	0.75
	Sni					

[a]Mean of 9 rats.

Table 39. F values for analyses of variance for Table 38

Experiment IV		
Measurement	Degrees of freedom for treatments	F
Spleen weight, gm	7	5.49**
Total spleen cell count x 10^7	7	9.42**
Nucleated cells, %	7	3.58**
Nucleated cell count x 10^7	7	6.85**
Plaque count/spleen, log	6	30.44**
Agglutinins, log	6	2.56*
Hemolysins, log	6	2.16[a]

[a] $P < 0.10$.

** Significant at $P < 0.01$.

* Significant at $P < 0.05$.

of antibody per cell was not affected by the balance of amino acids when diets contained the same amount of protein (R5 versus RM5 and RM7½ versus RAA7½). Apparently, protein deprivation caused a reduction in antibody-forming cells but did not significantly alter the ability of the cells to form antibodies. Groups RM7½, RAA7½ and Scol had highest AFC and antibody titers, RAA6½ was intermediate, and DD, R5 and RM5 had the lowest.

Table 40. Results of Duncan's test for Table 38[a]

Experiment IV

Group	DD	R5	RM5	RM7½	RAA7½	RAA6½	Sni	Scol
Spleen weight, gm	.47	.51	.57	.63	.64	.65	.66	.70
P < 0.01								
P < 0.05								

Group	DD	R5	RM5	RAA6½	RM7½	RAA7½	Scol
Plaque count, log	1.39	1.39	1.66	1.86	1.99	2.17	2.26
P < 0.01							
P < 0.05							

Group	R5	DD	RM5	RAA6½	Scol	RM7½	RAA7½
Agglutinins, log	1.00	1.16	1.20	1.32	1.54	1.60	1.76
P < 0.01							
P < 0.05							

[a]Any means not underscored by the same line are significantly different.

Table 40 (Continued)

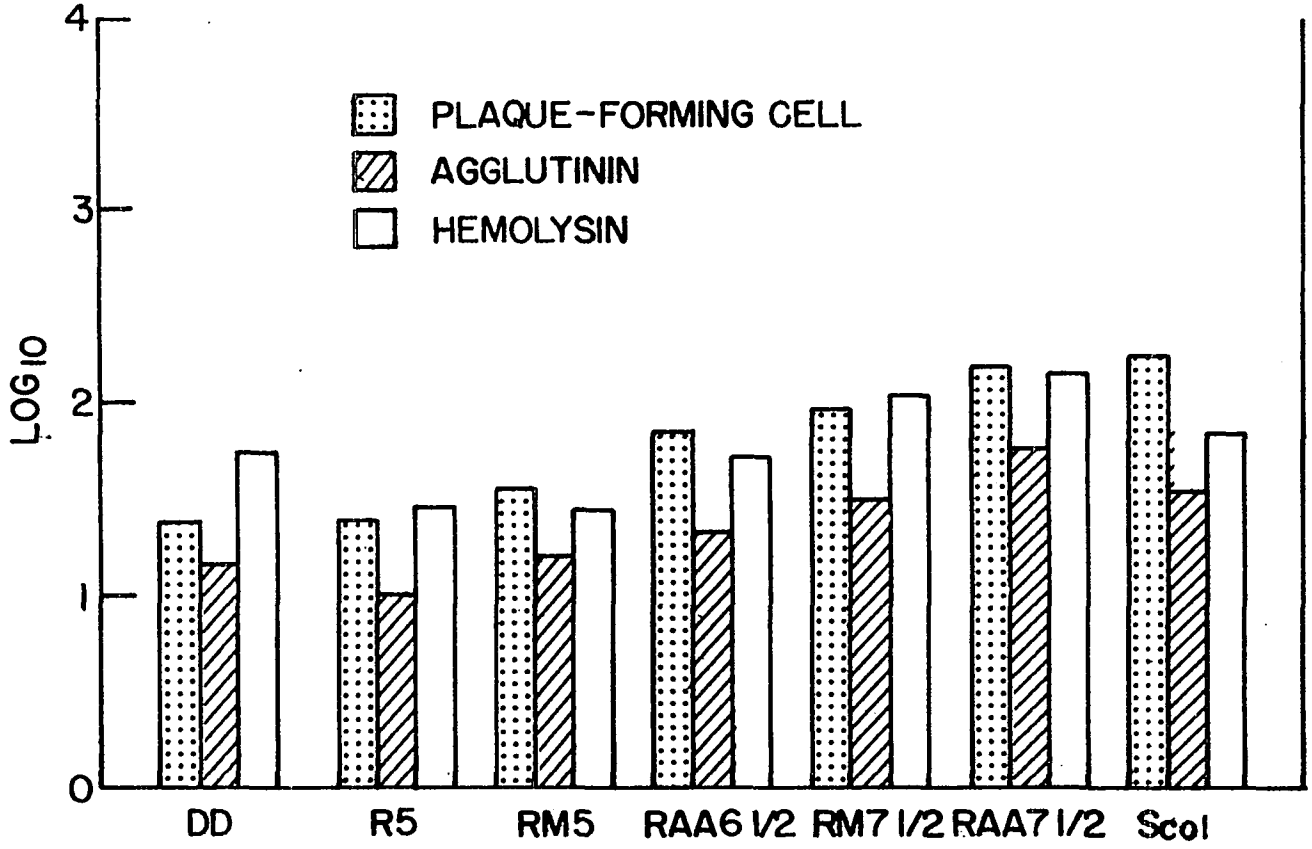
Group	DD	Scol	Sni	RAA7½	RAA6½	R5	RM5	RM7½
Nucleated cells, %	32.30	48.10	53.00	53.89	56.09	56.76	56.90	57.53
P < 0.01		———						
P < 0.05		- - -						

Group	R5	RM7½	DD	RM5	RAA7½	Sni	RAA6½	Scol
Total cell count x 10^7	52	54	56	57	67	70	75	146
P < 0.01	———							
P < 0.05	- - -							

Group	DD	R5	RM7½	RM5	RAA7½	Sni	RAA6½	Scol
Nucleated cell count x 10^7	17	28	31	31	37	38	43	66
P < 0.01	———	———						
P < 0.05	- - -	= =						

Group	R5	RM5	DD	RAA6½	Scol	RM7½	RAA7½
Hemolysins, log	1.48	1.55	1.68	1.73	1.81	2.03	2.11
P < 0.01	———	———					
P < 0.05	- - -	= = =					
P < 0.10	-·-·-	= = =					

Figure 3. Mean plaque counts, agglutinins and hemolysins - Experiment IV


PLAQUE-FORMING CELL
AGGLUTININ
HEMOLYSIN
LOG10
4
3
2
1
0
DD
R5
RM5
RAA6 1/2
RM7 1/2
RAA7 1/2
Scol

Animals on the stock ration had highest spleen weights, highest cell counts and largest number of nucleated cells per spleen. Even though depleted animals had lowest spleen weights, mean cell count per spleen did not differ from that of the experimental groups. The smallest number of nucleated cells per spleen was found in depleted rats. Neither cell count per spleen nor mean percentage of nucleated cells differed among experimental groups. There was also no difference in spleen weight among groups RAA6½, RM7½ and RAA7½. Even though spleen weight of R5 was significantly lower than that of these three groups ($P < 0.05$), the percentage of nucleated cells did not differ. Parameters indicative of antibody formation, namely plaque count, agglutinin and hemolysin titers, all were low in group R5 while there was no indication of a significant depression in the number of nucleated cells. This may be interpreted to mean that a relatively small fraction of the nucleated cells responded to the stimulus caused by the antigen and consequently were capable of synthesizing antibodies.

The percentage of nucleated cells was relatively constant among groups given the experimental diets and stock ration. However, in depleted rats, nucleated cells decreased more rapidly than total cells. Depletion reduced total cell count almost to 1/3 that of controls while the number of nucleated cells dropped to approximately 1/4.

Total cell count of immunized, stock-fed rats was approximately twice that of non-immune rats but the percentage of nucleated cells was not different. Therefore, one might infer that nucleated cells almost doubled with immunization.

Gross Observations

Rats given a stock ration continuously seemed to have more abdominal fat than did those depleted and subsequently repleted on the stock diet (Sr). In spite of a four-week depletion regimen, similar amounts of fat were found in both groups DD and Scol in Experiment I. This was confirmed in Experiment IV. In Experiments II and III smaller fat pads were noted in rats fed C6 or stock rations than in those fed R6 or the 10% supplemented diets. There appeared to be more fat in groups receiving RAA6½, RM7½ and RAA7½ than in groups fed R5 and RM5 (Table 41).

Fifteen percent of rats in Experiment I, 10% in Experiment II, 7% in Experiment III and 15% in Experiment IV had lung infections. There was no apparent relation between this finding and diet or various measures of protein adequacy.

Two of the rats (nos. 1 and 55) given diet R5 had oddly shaped spleens. Both spleens were constricted toward one end so that there appeared to be a large and a small portion. The immune responses of these two rats were not different from those of other rats in the group (R5).

Table 41. Gross observations

Experiment	Group	No. of rats	Abdominal fat[a]	Lung abnormalities[b] no.	Left testis weight gms	No. of deaths
I	Sr	12	1.8 ± 0.2[c]	4	1.9 ± 0.06	0
	Scly	12	2.7 ± 0.2	1	2.0 ± 0.04	0
	Scol	12	1.5 ± 0.2	2	2.0 ± 0.05	0
	DD	12	1.2 ± 0.1	0	1.8 ± 0.05	0
II	C6	10	1.6 ± 0.2	0		1[d]
	CF10	10	2.4 ± 0.2	1		2
	RCE10	10	2.8 ± 0.3	0		3
	R6	10	2.2 ± 0.4	0		2
	RF10	10	3.1 ± 0.2	2		0
	Sc	10	1.7 ± 0.2	1		1
	Sd	10	1.3 ± 0.2	2		1
IIIa	C6	10	1.1 ± 0.1	1		0
	CF10	10	1.7 ± 0.1	0		0
	RCE10	10	1.8 ± 0.1	0		0
IIIb	R6	10	1.5 ± 0.2	0		0
	RF10	10	1.9 ± 0.2	1		0
	Sr	10	1.3 ± 0.2	2		0
IV	R5	12	1.9 ± 0.3	3	1.7 ± 0.09	0
	RM5	12	2.0 ± 0.2	1	1.9 ± 0.08	0
	RAA6½	12	2.9 ± 0.2	1	1.8 ± 0.06	0
	RM7½	12	3.2 ± 0.3	3	1.9 ± 0.11	0
	RAA7½	12	3.2 ± 0.2	2	2.0 ± 0.09	0
	Scol	12	1.9 ± 0.1	1	1.9 ± 0.05	0
	DD	10	1.3 ± 0.2	1	1.8 ± 0.15	0
	Sni	12	1.8 ± 0.2	1	1.7 ± 0.05	0

[a]Graded 1, 2, 3, 4.

[b]Only those with definite lung infection characterized by pus sacs or numerous black spots.

[c]Mean ± S. E.

[d]Experiment II - Salmonela 0 antigen; all others Srbc.

In a previous study in this laboratory there had been some evidence that testicular weight changed with various quantities of dietary protein; hence, in Experiments I and IV, the left testis was weighed. Apparently, neither depletion nor repletion affected testicular weight of rats in these two experiments.

None of the rats given intravenous injection of Srbc died during the experiment; however, in Experiment II, with Salmonella O antigen, some rats, without any apparent relation to dietary treatment, died within several hours after the injection. A dose of one ml of a 1:100 dilution of a commercial preparation of antigen was given to group Sd while one ml of a 1:10 concentration was injected into all other groups. Dilution of the antigen did not prevent death.

Dietary Amino Acid Pattern

The amounts of essential and non-essential amino acid intakes in each of the experimental diets, calculated from analyzed values of the protein sources, are given in Tables 5 and 6. Mean intakes of each group take into account variations in the quantity of amino acid related to food intake per day (Tables 42 and 43).

Intakes were compared to daily minimum essential amino acid requirements for repletion of the protein-depleted rat. Requirements were estimated from data reported by Steffee et al. (1950). Calculations for repletion as presented by the

Table 42. Mean essential amino acid intake - Experiments II, III and IV

Experiment	Group	% protein	Lysine	Histidine	Tyrosine + Phenyl alamine	Tryptophan	sulfur-containing amino acid cystine & methionine
			mg	mg	mg	mg	mg
II	R	6	15.3	41.9	95.2	22.3	31.9
	C	6	18.2	36.4	68.5	7.8	31.3
	RF	10	76.8	62.9	149.9	32.6	82.6
	CF	10	80.8	64.6	136.9	20.1	86.6
	RCE	10	81.3	73.0	169.2	33.2	75.4
III	R	6	15.4	42.1	95.5	22.4	32.0
	C	6	21.4	42.9	80.7	9.2	36.8
	RF	10	170.9	67.1	160.0	34.8	88.1
	CF	10	85.4	68.2	144.5	21.2	91.5
	RCE	10	85.1	76.4	177.1	34.8	78.9
IV	R	5	11.9	32.5	73.8	17.3	24.7
	RM	5	20.4	33.8	79.9	18.0	22.5
	RAA	6½	53.9	52.9	113.6	22.7	39.5
	RAA	7½	69.0	57.1	139.9	23.0	42.7
	RM	7½	33.5	56.1	132.5	29.9	37.5

Table 42 (Continued)

Experiment	Group	% protein	Threonine mg	Leucine mg	Isoleucine mg	Valine mg	Total EAA
II	R	6	40.7	95.5	45.1	65.8	453.7
	C	6	32.7	115.5	31.1	42.6	384.1
	RF	10	75.8	160.2	79.3	107.0	827.1
	CF	10	73.7	201.5	71.2	92.2	827.6
	RCE	10	87.8	206.7	93.3	121.2	941.1
III	R	6	40.9	96.0	45.3	66.1	455.7
	C	6	38.5	136.2	36.7	50.2	452.6
	RF	10	80.9	120.9	84.6	114.2	882.5
	CF	10	77.9	212.8	75.1	97.4	874.0
	RCE	10	91.9	216.4	97.6	126.8	985.0
IV	R	5	31.6	96.0	35.0	51.0	351.9
	RM	5	33.7	81.3	40.1	53.3	383.0
	RAA	6½	54.9	121.8	64.6	86.2	610.1
	RAA	7½	60.4	132.2	72.2	93.8	690.3
	RM	7½	55.9	134.8	66.4	88.4	635.0

Table 43. Mean non essential amino acids intake - Experiments II, III and IV

Experiment	Group	% protein	Glutanine mg	Glycine mg	Alanine mg	Arginine mg
II	R	6	218.6	35.6	77.9	86.6
	C	6	141.5	22.5	81.3	38.0
	RF	10	321.7	94.9	130.4	139.7
	CF	10	274.0	84.6	149.0	99.1
	RCE	10	326.4	65.3	149.0	124.1
III	R	6	219.5	35.8	78.3	87.0
	C	6	166.8	26.5	95.8	44.8
	RF	10	343.2	101.2	139.2	149.1
	CF	10	289.4	89.4	157.4	104.7
	RCE	10	341.6	68.3	155.9	129.8
IV	R	5	169.6	27.6	60.5	67.2
	RM	5	181.8	30.0	59.5	67.5
	RAA	6½	199.4	43.6	75.0	115.2
	RAA	7½	191.7	46.8	73.8	126.4
	RM	7½	301.4	49.8	98.8	112.1

Table 43 (Continued)

Experiment	Group	% protein	Serine mg	Proline mg	Aspartic acid	Total non-eaa
II	R	6	63.1	56.3	124.9	663.0
	C	6	68.6	80.4	70.3	502.6
	RF	10	95.6	95.4	200.6	149.7
	CF	10	114.2	134.2	160.2	1015.3
	RCE	10	144.3	116.1	208.3	1133.5
III	R	6	63.4	56.5	125.4	665.9
	C	6	80.9	94.7	82.8	592.3
	RF	10	102.0	101.8	214.0	1150.5
	CF	10	120.6	141.7	169.2	1072.4
	RCE	10	151.0	121.5	218.0	1186.1
IV	R	5	49.0	43.6	96.9	514.4
	RM	5	53.1	46.5	114.2	552.6
	RAA	6½	55.8	49.8	110.4	649.2
	RAA	7½	52.9	47.2	104.7	643.5
	RM	7½	88.0	77.1	189.1	916.3

authors were extrapolated from requirements of the 150-gm rat.

Lysine appeared to be first limiting amino acid in all diets except CF10. Isoleucine and sulphur-containing amino acids seemed to be second limiting factors in these diets except diet C6. Aside from isoleucine, tryptophan appeared to be limiting in diet C6. Sulphur-containing amino acids, lysine and isoleucine appeared to be equally limiting for RAA7½.

The addition of fish to corn or rice increased lysine content in diets RF10 and CF10. Although fish increased lysine content of diet RF10, lysine remained limiting. Isoleucine became first limiting in diet CF10. Both lysine and tryptoplan were low in diet CF10 while threonine was low in diet RF10.

Benditt et al. (1950) used the minimum requirements for maintenance and repletion of the adult depleted rat to calculate minimum requirements of humans. They compared the values obtained to minimum requirements observed on humans by Harte and Rose. Benditt and co-workers (1950, pp. 347-348) commented that "the phenylalanine requirement as estimated from the rat data is quite low and the isoleucine estimate is rather high compared with the estimates from observations on humans".

Thus, due to high isoleucine requirement, isoleucine appeared to be the first limiting amino acid when rice or corn was supplemented with fish or egg. Whether this is an error in estimating isoleucine requirement of the rat or a true species difference remains to be determined.

DISCUSSION

Protein synthesis occurs in the nucleus, mitochondria and ribosomes of the cell (Lust, 1966). It is especially active in liver, gut, muscles, spleen and other organs. Various mechanisms in the cell maintain a constant internal environment. However, changes in the external environment of the cell influence cellular metabolic processes. Protein nutriture is only one of the multifarious factors that can cause metabolic changes within the cell (Svoboda and Higginson, 1964). Proper amounts of amino acids, a source of energy and ribonucleic acids are some factors necessary for protein synthesis. Dietary alterations in quantity or quality of protein or length and/or severity of nutrient deprivation may influence the internal processes of the cell (Allison et al., 1963). Hence, the intracellular pattern of amino acids and the ability of cells to synthesize proteins depends partly on the extracellular environment.

Repletion of different body tissues does not occur at the same rate (Addis et al., 1936; Neuberger and Richards, 1964). Although changes in body weight, hepatic nitrogen and lipids are some common measurements employed for the assessment of the adequacy of dietary proteins and of protein nutriture, measurements of hepatic nucleic acids, antibody-forming cells of the spleen and circulating antibodies might help discern subtle changes at the cellular level. These measurements

might show how and where dietary proteins influence antibody synthesis. The formation of "competent cells" requires protein even before circulating antibodies are formed. Impaired synthesis of RNA and DNA in the spleen and liver, as has been observed, may inhibit antibody formation during protein deficiency (Trakatellis and Axelrod, 1965). Hence, these parameters were chosen for this study. It was hoped that by these measurements, antibody production by rats could be related to some specific dietary amino acid pattern. Previous studies in this laboratory (Glabais, 1946; Kenney, 1963; Smith, 1966; Williams, 1967) had shown that rats differ in their immune response to an antigen when fed various dietary proteins or deprived of protein.

Each dietary protein presents a different amino acid pattern for protein biosynthesis. At a given protein concentration the amino acid pattern of a diet is that characteristic probably responsible for differences in weight gains if other components of the diet are held constant (Winje et al., 1954). Even if the unprocessed protein source of a diet seems excellent when compared to the minimum amino acid requirements of a certain species or to some reference protein, factors such as subsequent heat treatment, digestibility, and rate of absorption may affect the availability of the amino acids in the protein source. Various rates of liberation of amino acids from dietary proteins in the gastro-intestinal tract may alter the amounts of amino acids available for absorption at a given

time. For example, certain of the amino acids in zein are only partially available to the rat (Geiger et al., 1952). Also, if isolated amino acids are used, availability may vary. For instance, the need for sodium bicarbonate in diets containing the hydrochloride forms of arginine, histidine and lysine was demonstrated by Breuer et al. (1964). These are some of the factors responsible for the differences observed between utilization of amino acid in intact protein or isolated forms.

Sauberlich (1961) stressed the point that "amino acid requirements of the rat are not constant factors but are related to the diet used and, in particular, to the amino acid balance and protein or nitrogen level of the diet," (p. 304). One may consider two major facets of amino acid balance: one concerns the ratio of essential to non-essential amino acid and the other deals with the balance of essential amino acids.

Agreement is lacking with respect to an optimum ratio of essential to non-essential amino acid. Rosenberg (1959) suggested that equal quantities of essential and non-essential amino acids would promote good growth, while Frost and Sandy (1951) believed that around 20-30% of the nitrogen should come from non-essential amino acids. Rose et al. (1948) obtained better growth response in young rats with a mixture of 19 amino acids than with one of 10 essential amino acids plus glutamic acid. A mixture of non-essential amino acids similar

to that of casein was used by Rama Rao et al. (1960), Sauberlich (1961) and Breuer et al. (1964). All these investigators reported that rats ate and grew better when dispensable amino acids were included in the diet than with essential amino acids alone.

In the present study, almost all the experimental diets had approximately 40% of essential amino acids, with the exception of diets RAA6½ and RAA7½. These had about 49% and 54%, respectively, of total amino acids in the form of essential amino acids (Table 5).

On the assumption that the non-essential amino acids were adequately supplied in the rations used, an evaluation of the essential amino acids of each diet in terms of rat requirement was carried out. The relationship of the essential amino acid patterns to various parameters will be discussed.

Weight Gains and Nitrogen Efficiency Ratio

Generally, there was an increase in body weight as well as organ weights upon repletion with the various experimental diets. However, weight gains varied. The failure of rats on the rice or corn regimen to regain lost body weight during the experimental period may be attributed either to the low total nitrogen intake or to an imbalance of amino acids or to both.

Nitrogen efficiency ratio of diets R6 and C6 differed significantly. A comparison of the amino acid intake to the minimum requirements (Steffee et al., 1950) for an adult

depleted rat for maintenance and repletion showed that lysine was deficient in most of the diets. In the rice diet with 6% protein, 11.7% of the lysine requirement was met whereas diet C6 supplied 16.2% of the lysine requirement, yet, diet R6 produced greater weight gains than diet C6 (Table 44). It appears therefore, that lysine of the rice protein was better utilized than that supplied by corn assuming that composition data are exact.

Though lysine was the most deficient amino acid in both diets, the amino acid pattern of rice seems to be better than that of corn as judged from the nitrogen efficiency ratio. This confirms the reports of Sure (1957) whose investigations showed that the biological value of rice was higher than that of corn. If growth were proportional only to the amount of the limiting amino acid, namely lysine, diet C6, which had higher lysine content, should have promoted better growth than diet R6. Therefore, factors other than the single most limiting amino acid must have influenced the results. One of these may have been the leucine-isoleucine ratio. This ratio was about 3:1 in corn while it was approximately 2:1 in rice; the requirements are in a ratio 1.2:1. Sauberlich (1961), using pure amino acid mixtures in the diet of weanling rats, observed growth depression when isoleucine in the diet was kept constant while increasing amounts of leucine at leucine-isoleucine ratios of 1:1, 4:1 or 8:1 were added to the diets. Increasing

Table 44. Percentages of requirements of essential amino acids for repletion furnished by various diets[a]- Experiments II, III and IV

		Lysine	Histidine	Phenylalanine and tyrosine	Tryptophan
		mg	mg	mg	mg
Requirement (mg/day)[b]		132	48	102	32
Experiment II	R6	11.6[c]	87.3	93.3	69.7
	C6	13.8	75.8	67.2	24.4
	RF10	58.2	131.0	147.0	101.2
	CF10	61.2	134.6	134.2	62.8
	RCE10	61.6	152.0	165.9	103.8
Experiment III	R6	11.7	87.7	93.6	70.0
	C6	16.2	89.4	79.1	28.8
	RF10	62.0	140.0	156.9	109.0
	CF10	64.7	142.0	141.7	66.0
	RCE10	64.5	159.0	173.6	108.8
Requirement (mg/day)[d]		165	60	128	40
Experiment IV	R5	7.2	54.2	57.7	43.2
	RM	12.4	56.3	62.4	45.0
	RAA6½	32.7	88.2	88.8	56.8
	RAA7½	41.8	95.2	109.3	57.5
	RM7½	20.3	93.5	103.5	74.8

[a]Protein sources analyzed for amino acids by Wisconsin Alumni Research Foundation Laboratory, Madison, Wisconsin.

[b]Estimated for the 400 gm protein-depleted rat from Steffee et al., 1950.

[c]Lowest percentage is underlined.

[d]Estimated for the 500 gm protein-depleted rat from Steffee et al., 1950.

Table 44 (Continued)

		Methionine + cystine mg	Threonine mg	Leucine mg	Isoleucine mg	Valine mg
Requirement (mg/day)		88	98	166	138	116
Experiment II	R6	36.2	41.5	57.5	32.7	56.7
	C6	35.6	33.4	69.5	22.5	36.7
	RF10	93.9	77.3	96.5	57.5	92.2
	CF10	98.4	75.2	121.0	51.6	79.5
	RCE10	85.7	89.6	124.5	67.6	104.5
Experiment III	R6	36.4	41.7	57.8	32.8	57.0
	C6	41.8	39.3	82.0	26.6	43.3
	RF10	100.0	82.6	103.0	61.3	98.4
	CF10	104.0	79.5	128.2	54.4	84.0
	RCE10	89.7	93.8	130.4	70.7	109.0
Requirement (mg/day)		110	122	208	172	145
Experiment IV	R5	22.4	25.9	35.6	20.3	35.2
	RM	20.5	27.6	39.1	23.3	36.8
	RAA6½	35.9	45.0	58.6	37.6	59.4
	RAA7½	38.8	49.5	63.6	42.0	64.7
	RM7½	34.1	45.8	64.8	38.6	61.0

the level of isoleucine prevented growth depression due to high leucine. Another factor responsible for differences in weight gain may have been the isoleucine supply of the two diets. This amino acid, though calculated to be second limiting in both diets was present in larger amounts in diet R6 as compared to diet C6.

Besides lysine and perhaps isoleucine, corn is deficient in tryptophan. This may be another amino acid that has caused differences in weight gain between groups R6 and C6. Diet R6 contained 70% of the required tryptophan whereas diet C6 met only 29% of the tryptophan requirement.

When rice or corn was supplemented with fish, which is high in lysine, or when a mixture of three proteins (rice, corn and egg) was given as 10% of the diet, the amount of lysine as well as other essential amino acids was increased and nitrogen intake was also raised. Consequently, repletion of depleted tissues was more rapid than with 6% protein. With fish supplementation, the ratio of isoleucine to leucine as found in rice was not altered. In the corn diets, the ratio was reduced from 3:1 (C6) to 2.5:1 (CF10). The ratio was 2:1 in the rice, corn, egg combination.

With fish supplementation, lysine and isoleucine became equally limiting in diet RF10 while isoleucine and tryptophan became limiting for diet CF10. Diet RF10 met about 2/3 of the isoleucine and lysine requirements whereas diet CF10 met only approximately half of the isoleucine requirement and 2/3 of

the lysine and tryptophan requirements. Thus, the difference in amino acid pattern is probably one of the causes for the higher NER of diets R6 and RF10 as contrasted to diets C6 and CF10. Fish supplementation improved the NER of diet C6 but did not alter that of diet R6 which had produced NER values as high as those related to the two supplemented rice diets.

The requirement for tryptophan was met in diet RF10 whereas diet CF10 contained only 2/3 of the requirement for tryptophan. Rosenberg et al. (1960) noted maximum weight gains in weanling rats fed white corn meal supplemented with 5.5 parts of lysine to 1 part of tryptophan compared to rats on a basal diet containing 90% corn meal. The authors were not able to pinpoint a third limiting essential amino acid. Methionine, threonine, isoleucine and valine were equally third limiting in weanlings when requirements for lysine and tryptophan were met. In this study, using Steffee's estimates of need for repletion, lysine would be first limiting in corn, while tryptophan and isoleucine would be equally limiting when lysine requirements are met by supplementation. Threonine appeared to be third limiting.

In the present study mung supplementation or substitution increased lysine content of the diets but lysine remained limiting. Sulphur-containing amino acids and isoleucine were also low in the rice-mung diets (RM5, RM7½) as well as in the rice-amino acid combinations (RAA6½ and RAA7½).

The nitrogen efficiency ratio of mung beans has been found to be lower than that of rice (Phansalkar et al., 1957) but because of the bean's high nitrogen content this legume has been considered a good supplement to increase protein intake. The authors reported a NER ratio of 12.62[1] for rice and 9.49 for mung beans at 10% level of protein intake. In the present study, NER was the same for diets R5 and RM5 and RM7½ since differences in values were not statistically significant. Although mung substitution did not improve NER of rice, mung supplementation was beneficial, since it was possible to raise the nitrogen intake. Supplementation with isolated amino acids comparable to those in mung beans (diets RAA6½, 7½) improved NER at the 6½ but not at the 7½% protein intake. Since NER tends to drop after a certain level of protein intake is reached, it is possible that the 6½ level was maximum for rice supplemented with these particular amino acids. The inclusion of a rice and mung bean combination at 6½ protein would have been helpful in verifying this assumption.

It is widely recognized that amino acid deficiencies other than those of the most limiting amino acid may be of great importance (Harper et al., 1955; Pecora and Hundley, 1951). Furthermore, what has been found to be limiting by

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[1]Recalculated from protein efficiency ratio using a factor of 6.25.

calculation may not always be the most deficient. Pecora and Hundley (1951) compared the essential amino acids of a 90% cooked rice diet to requirements for growth as reported by Rose and found that the order of deficiency of essential amino acids was lysine, histidine, methionine and threonine. Lysine was shown to be limiting from their calculations, but in their experiments, threonine was as limiting as lysine. When the diet of weanling rats was supplemented with lysine alone, the protein efficiency ratio of the rice diet was not improved. Studies of Pecora and Hundley (1951) as well as those of Harper et al. (1955), Rosenberg et al. (1959) and Parthasarathy et al. (1964) showed that supplementation of rice with lysine and threonine together improved the protein efficiency ratio of rice. Supplementation with either one failed to improve protein efficiency.

A direct comparison between other investigations and the present study cannot be made, because in this report, adult depleted rats were used whereas other researchers used weanling rats. Ratios of essential amino acids show that lysine and phenylalanine requirement for growing rats are about twice that for an adult depleted rat while requirement for sulphur-containing amino acid is a third more for the weanling than for the adult depleted rat (Table 45). One should also be cautious in interpreting data using natural sources of protein, since amounts and proportions of amino acids may differ in varieties or in samples of the same variety of intact protein

Table 45. Ratios of daily minimum essential amino acid requirements for growth and for an adult depleted rat for maintenance and repletion based on tryptophan = 1.00

	Minimum requirement for growth[a]	Minimum requirement for maintenance and repletion[b]
Histidine	2.3	1.5
Lysine	8.2	4.1
Tryptophan	1.0	1.0
Isoleucine	5.0	4.3
Valine	5.0	3.6
Leucine	6.4	5.2
Threonine	4.5	5.2
Methionine + cystine	4.5	2.8
Phenylalanine + tryosine	6.5	3.2

[a]Rama Rao et al., 1964.

[b]Steffee et al., 1950.

sources. However, indirectly, and with reservations, some comparison may be made with reports in the literature since it has been well established that rice and corn are deficient in lysine based on several standards of reference.

Hepatic Lipid and Nitrogen

Several dietary factors have been linked to fatty infiltration of the liver. Some of those pertinent to this discussion are low protein intakes, unsuitable dietary amino acid patterns and inadequacies in choline, methionine, threonine, lysine and isoleucine. Vitamin and mineral supplements were given daily and it was assumed that the amount of choline supplied in the diet was adequate.

Generally, hepatic lipids decreased while hepatic nitrogen increased with increasing amounts of protein in the diet. Not only the rise in nitrogen intake but also changes in amino acid composition of the diet affected liver fat and nitrogen concentrations. The fact that hepatic lipid in rats fed rice alone (R6) was higher than that of rats fed corn as the sole source of protein (C6) would suggest that the higher concentrations of hepatic lipid in rats fed rice was due to an amino acid deficiency or an imbalance of the amino acid composition. This confirms the report of Harper and co-workers (1955). They observed less fat in livers of animals fed corn than in those fed rice.

Lysine, threonine and sulphur-containing amino acids such as methionine and cystine have been found to be lipotropic agents. As was previously mentioned, although lysine was limiting in rice or corn diets, it was lower in rice than in corn. Sulphur-containing amino acids were also lower in the rice than in the corn diet. Not only was the lysine content of the

diet increased by fish supplementation or addition of corn and egg to rice but the supply of sulphur-containing amino acids was improved also, so that about 100% of the requirement of these amino acids was met. With supplementation threonine was also doubled in both diets. Thus, hepatic fat dropped to levels similar to those of rats fed the stock ration. Since methionine has been shown to be deficient in mung beans, substitution with mung did not improve total sulphur-containing amino acid of rice in diet RM5. However, hepatic lipid concentration was significantly decreased. Except for lysine, the essential amino acids of diets R5 and RM5 were essentially similar. Mung substitution raised the lysine content of diet RM5. Supplementing rice with mung in diet RM7½ increased total nitrogen as well as all the essential amino acids but there was no further change in amino acid balance. This may account for the failure to lower hepatic fat concentration when protein was increased from 5 to 7½. When rice was supplemented with amino acids to give the same 7½% protein (RAA7½), lysine, sulphur-containing amino acids, isoleucine and threonine were increased to a level exceeding all other diets studied in Experiment IV. The lowest value for hepatic lipids was found with diet RAA7½ while hepatic nitrogen concentration was highest. The reverse was true for group R5, where the concentration of fat was greatest and the percentage of nitrogen the smallest. This diet had the lowest lysine content and furthermore, was low in sulphur-containing amino acids. Therefore, in the mung

experiment, amino acid imbalance was likely responsible for the hepatic fatty infiltration of the liver in all experimental groups except RAA7½.

Likewise, in the mung bean experiment, lysine was most limiting and total sulphur-containing amino acids also appeared to be deficient in all diets; at the same time livers showed fatty infiltration in all experimental groups except group RAA7½.

Nucleic Acids

The ratio of RNA to DNA has been used to estimate changes in RNA concentration at the cellular level. In this study, RNA/DNA ratios increased with increasing amounts of nitrogen and/or improvement in amino acid composition of the diet. This confirms reports of Allison and co-workers (1962), Svoboda et al. (1966) and Banks et al. (1964) that RNA relative to DNA increased with the adequacy of the amino acids in the diet. The relative constancy of hepatic total DNA has induced investigators to interpret changes in RNA/DNA ratio as an index of RNA concentration per cell.

In this study, total hepatic RNA (Experiment III) was higher in supplemented rats than in those fed the unsupplemented diets, indicating that with increased intakes and qualities of protein, protein biosynthesis was intensified. This was confirmed by the increase in protein per cell which was reflected by protein/DNA ratio. Just like total hepatic RNA,

protein/DNA was higher in supplemented groups than in those fed the unsupplemented diets. Protein/RNA was not significantly different. Since the RNA concentration of CF10 differed significantly from that of RF10, one would surmise that the amino acid composition of the diet was also partly responsible for the difference in total RNA.

Mung bean protein substitution or supplementation increased total hepatic RNA just as fish supplementation of rice had raised this parameter in the liver. Total DNA was independent of nitrogen intake and amino acid composition. RNA/DNA ratios however, were not significantly different among the experimental diets in Experiment IV. This seems to indicate that protein biosynthesis in the liver was not enhanced since there was no improvement in RNA/DNA ratio with mung bean or amino acid supplementation.

Several investigators (Allison et al., 1962; Campbell and Kosterlitz, 1952) have reported that total hepatic DNA is independent of nitrogen and/or improvement in amino acid composition of the diet. Results of this study also showed that total DNA was relatively constant and was independent of nitrogen intake. Although total DNA was higher in groups C6, CF10 and RCE10 than in groups R6 and RF10 (Experiment III), the results may have been influenced by experimental conditions rather than by dietary variations since the experiments with corn-fish diets were not carried out simultaneously with the rice and rice-fish diets. Livers of rats of groups R6 and RF10

were homogenized and frozen a year before they were analyzed for nucleic acids, whereas those of groups C6, CF10 and RCE10 were freshly homogenized from the frozen state at the time of analysis.

When a comparison of RNA/DNA ratios with NER is made, it will be noted that fish supplementation augmented both RNA/DNA ratio and NER but the effect of supplementation did not present a similar pattern for both measurements. The NER and RNA/DNA ratio were similar for R6 and RF10. Unlike R6 and RF10, CF10 and RCE10 had significantly different NER but RNA/DNA ratios were similar. In contrast, hepatic RNA/DNA ratios were similar among the experimental groups in experiment IV. The NER showed that nitrogen utilization was significantly enhanced only in diet RAA6½. From these data it is not possible to state which one of these measurements is the more sensitive.

Immune Response

The relation of the concentration of circulating antibodies (agglutinins and hemolysins) to number of antibody-forming cells of the spleen has been reported by several investigators (Biozzi et al., 1968; Jerne et al., 1963; Hege and Cole, 1966a; Kenney et al., 1968). The spleen is not only composed of antibody-forming cells, but also of other cells not related to antibody formation. Furthermore, the spleen is not the only site for antibody production; other cells of the reticulo-endothelial system are capable of producing anti-

bodies. The degree to which numbers of antibody-forming cells can be related to amount of circulating antibody is limited. However, there is evidence which points to some relationship between spleen antibody-forming cells and circulating antibodies in the presence or absence of dietary protein (Kenney et al., 1968).

The decreases in total number of cells per spleen, number of nucleated cells, titers of agglutinins and hemolysins in protein-depleted rats (groups DD, Experiments I and IV) suggest that the spleen was sensitive to protein deprivation (Tables 31, 38). Generally, antibody-forming cells of the spleen, nucleated spleen cells, and circulating antibodies tended to increase upon repletion.

A concomitant increase in circulating antibodies was observed with an increase in plaque counts and with increased nitrogen intake as well as with substitution or supplementation in Experiment IV (Table 38). There was a highly significant positive relationship between plaque counts and circulating antibody titers based on means of all groups in Experiment IV (Table 46) but not in Experiment III. Although depleted rats had the fewest antibody-forming cells, the ability of these cells to form agglutinins and hemolysins was not significantly impaired (Tables 38, 40). Antibody titers of the depleted group did not differ from those of animals fed R5 and RM5 or RAA6½. Thus, the ability of plaque-forming cells to form antibodies did not seem to be greatly altered in

Table 46. Coefficients of correlation for circulating antibodies and antibody-forming cells of the spleen in depleted and refed animals - Experiments III and IV

Comparison	Experiment	Groups	Degrees of freedom n-2	Correlation coefficient[a]
Agglutinin vs. hemolysin	III	all groups	4	0.021
	IV	all groups	5	0.960**
Agglutinin vs. AFC	III	all groups	4	0.053
	IV	all groups	5	0.910**
Hemolysin vs. AFC	III	all groups	4	0.071
	IV	all groups	5	0.770*
Weight gain vs. AFC	III	all groups	4	0.003
	IV	R5, RM5, RAA6½, RAA7½, RM7½	3	0.840

[a]Probabilities less than 0.05 are designated by *; probabilities less than 0.01 by **.

protein depletion under the conditions of the experiment. The decline in antibody titers in protein deficiency may be ascribed to the reduced number of antibody-forming cells in the spleen rather than the ability to form circulating antibodies. Antibody titers of rats tended to increase with increased nitrogen intake and with mung bean or amino acid supplementation.

It is possible that the lack of correlation between plaque counts and circulating antibodies in Experiment III was not a function of amino acid pattern of diets but rather may have been due to two factors: 1) Experiment IIIa (groups C6, CF10 and RCE10) and Experiment IIIb (groups R6, RF10 and Sr) were not carried out simultaneously; 2) improvement in agar-plaque technique in Experiment IIIb.

Higher antibody titers might have been obtained if the mung beans had not been autoclaved. This suggestion is made on the basis of work by Chitre and Vallury (1956). According to these authors, autoclaving of the beans improved growth but globulin, which contains antibody, was diminished from 1.93 gm/100 ml to 1.31 gm/100 ml. Total plasma protein was also decreased from 3.80 gm/100 ml to 2.76 gm/100 ml when the beans was autoclaved.

Weight gain on repletion did not always parallel plaque counts and circulating antibody titers (Tables 3, 38). This confirms reports of Smith (1966) and Kenney (1963) that circulating antibodies were not always the highest where weight

gain was highest. Williams (1967) also failed to demonstrate any relationship between weight gain and antibody formation. Probably the amino acid pattern that seems best for growth is not always the best for antibody production. Furthermore, some amino acids seem to affect antibody-formation more than others. Gershoff and co-workers (1968) were able to demonstrate the necessity for tryptophan and phenylalanine but not methionine in antibody formation. Results in the present study suggest that some proteins are perhaps preferentially used for repletion of tissues rather than for antibody formation.

In Experiment III, it is difficult to state with certainty which of the 10% protein diets was superior for both repletion and immune response. Groups RCE10 and RF10 showed the best performance in terms of weight change and NER while CF10 was relatively lower in the scale, although the differences were not significant in most of the parameters measured. There were no significant differences in circulating antibodies and AFC counts. Effects of dietary alterations on antibody titers were inconsistent in Experiments II and III.

In Experiment IV, increase in nitrogen and improvement in amino acid composition of the diet increased AFC and circulating antibodies; liver nitrogen and RNA/DNA, were related with antibody-forming cells of the spleen and with circulating antibodies in the mung- or amino acid-supplemented rice diets. In stock (Scol) and depleted animals these measurements were also related to AFC, but not necessarily to hemolysin and agglutinins.

The stock ration was superior to all the other diets in repletion with respect to weight gain, liver nitrogen, liver fat and number of nucleated cells but not to circulating antibodies. Results suggest that proteins that may be recommended for repletion on the basis of gain induced in body weight may not necessarily enhance immune response.

SUMMARY

Immune and other responses of rats to various dietary protein sources were studied in a series of four experiments. Groups of 10-12 adult male rats weighing approximately 400-500 gms were depleted of protein for either 3 or 4 weeks and refed for 2 or 3 weeks. Rats given stock ration throughout or during the repletion period served as controls. Nitrogen sources of the experimental diets were rice or corn, alone or in combination with fish meal, egg, mung beans or free amino acids at protein levels ranging from 5 to 10%.

On the 8th day of repletion, one ml of a 1:100 or 1:10 dilution of Salmonella O antigen or 2% sheep red blood cells was injected into the caudal vein. The rats were bled on the 6th and sometimes 10th and 14th days after immunization. After two or three weeks of repletion, animals were sacrificed. Blood was collected for agglutinin and hemolysin determinations in serum. Livers were analyzed for total nitrogen, lipid, ribonucleic acid and deoxyribonucleic acid. Antibody-forming cells of the spleen were determined by the agar-plaque technique. The number of total spleen cells and nucleated spleen cells were also counted.

Animals had lost approximately 1/5 of their initial body weight at the end of the depletion period and upon refeeding regained varying amounts of the lost body, liver and spleen weights. Gain in weight during the repletion period was

dependent on the amount and kind of protein. When amino acid intakes were compared to daily minimum requirements for repletion, lysine appeared to be first limiting amino acid in all diets except in the 10 percent corn - fish diet which lacked isoleucine. Isoleucine and sulphur-containing amino acids seemed to be second limiting factors in these diets except the six percent corn diet (C6). Tryptophan appeared to be limiting in diet C6 aside from isoleucine. Total hepatic DNA content appeared to be independent of quality and quantity of protein. Hepatic nitrogen, total RNA and RNA relative to DNA tended to rise with increasing amounts of protein and improvement of the amino acid pattern of the diet. As was expected, an inverse relationship was observed between hepatic nitrogen and hepatic lipids.

Agglutinin and hemolysin titers of animals fed rice or corn or fish-supplemented diets or a combination of rice, corn and egg were not significantly altered by amount and kind of protein. Antibody-forming cells (AFC) were significantly higher in groups fed six percent corn, 10 percent corn - fish and 10 percent rice-corn-egg than those in groups that received six percent rice and stock ration. This difference may have been due to difference between experiments rather than due to a function of amino acid pattern. In groups of rats given rice with mung bean or amino acid substitution or supplementation, circulating antibodies and AFC count increased with dietary protein improvement. Positive correlations between

plaque counts and circulating antibodies and between agglutinins and hemolysins were discerned in the rice-mung experiment. Antibody formation was not always related to weight gain. RNA relative to DNA in liver seemed also to be positively related to antibody-forming cells of the spleen and to circulating antibodies with the rice, mung or amino acid supplemented diets.

From the variables studied no definite amino acid pattern emerged that could be considered the best for immune response. The amounts of circulating antibodies were a sensitive measurement for relating protein intake to immune response of rats. However, measurement of AFC may be a more sensitive indicator of some changes in the process resulting from changes in protein nutrition. Nevertheless, under the conditions of this experiment, the rice diet supplemented with amino acids (RAA7$\frac{1}{2}$) seemed to be the best among the experimental diets. Since the amounts of protein in the mung bean experiment were suboptimal, there is a possibility that the amino acids in the diet were utilized preferentially for repletion of tissues other than those producing circulating antibodies.

Rice was a better protein than corn in most respects. Because rice had a nitrogen efficiency ratio higher than that of corn to start with, fish meal was a relatively better supplement for corn than for rice. Other factors such as higher leucine:isoleucine ratio, as well as the low isoleucine supply in the six percent corn diet might have been responsible

for the differences in nitrogen efficiency ratio. Also, the six percent corn diet was deficient in tryptophan. It met only 29% of the tryptophan requirement whereas diet R6 contained 70% of the required tryptophan.

The combination of rice and fish (2 protein sources) proved to be as good as, if not better than, the combination of 3 proteins, rice, corn and egg. When mung beans increased nitrogen intake they were beneficial in raising the protein content of the diet, but they did not seem to alter the immune response appreciably at the low level of protein intake.

The stock diet was superior compared to all the other diets in all parameters measured except for circulating antibodies. Rats given the low-nitrogen diet did not always present the lowest amounts of circulating antibodies but were poorest in all other respects.

Under the conditions of the experiment, results suggest that proteins that may be recommended for tissue repletion may not necessarily be the best for enhancing antibody formation.

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APPENDIX

Table 47. Body weight initially, on day of repletion, at autopsy, food intake per day and total nitrogen intake of each rat - Experiments I, II, III & IV

Experiment	Group	Rat no.	Body weight			Food intake	Total nitrogen intake
			Initial	Day of repletion	Autopsy	day	
			gm	gm	gm	gm	gm
I	Sr	81	519	384	514	28	
		82	517	431	541	24	
		85	512	420	483	19	
		86	491	420	503	23	
		89	497	385	500	20	
		90	502	406	505	22	
		93	486	387	478	22	
		94	481	405	536	25	
		97	505	423	523	24	
		98	500	404	480	21	
		101	503	406	495	21	
		102	506	365	495	26	
	Mean ± S.E.		502 ± 3.35	403 ± 5.62	504 ± 5.98	23 ± .75	
	Scly	83	524		585	26	
		84	524		620	23	
		87	498		542	21	
		88	497		536	22	
		91	504		588	23	
		92	495		587	24	
		95	492		579	22	
		96	511		619	26	
		99	513		577	25	
		100	501		580	24	
		103	506		544	20	
		104	504		585	24	
	Mean ± S.E.		506 ± 3.04		578 ± 7.74	23 ± .52	

Table 47 (Continued)

Experiment	Group	Rat no.	Body weight			Food intake	Total nitrogen intake
			Initial	Day of repletion	Autopsy	day	
			gm	gm	gm	gm	gm
I	Scol	117	492		480		
		118	482		451		
		119	494		490		
		120	475		485		
		121	517		508		
		122	480		471		
		123	486		478		
		124	472		469		
		125	505		490		
		126	480		472		
		127	486		470		
		128	483		470		
	Mean ± S.E.		488 ± 3.70		478 ± 4.15		
	DD	105	489		389	17	
		106	492		415	18	
		107	506		392	16	
		108	504		404	16	
		109	497		433	17	
		110	505		395	15	
		111	491		386	16	
		112	508		396	12	
		113	509		395	13	
		114	504		401	13	
		115	493		416	16	
		116	505		395	14	
	Mean ± S.E.		500 ± 2.10		401 ± 3.92	15 ± .54	

Table 47 (Continued)

Experiment	Group	Rat no.	Body weight: Initial gm	Body weight: Day of repletion gm	Body weight: Autopsy gm	Food intake / day gm	Total nitrogen intake gm
II	R6	821	458	366			
		826	428	355	411	21	4.42
		831	473	392			
		836	415	354	386	17	3.55
		841	429	357	406	20	4.27
		846	432	349	448	27	5.68
		851	412	346	452	28	5.93
		856	435	344	407	25	5.36
		861	440	363	468	27	5.69
		866	410	333	401	25	5.32
	Mean ± S.E.		425 ± 3.99	356	422 ± 10.38	24 ± 1.4	5.03 ± .30
	C6	822	452	361	358	18	3.79
		827	431	346			
		832	424	353	378	21	4.33
		837	428	360	398	21	4.37
		842	408	339	338	16	3.44
		847	441	357	401	22	4.56
		852	425	347	372	21	4.30
		857	424	345	374	20	4.28
		862	426	341	356	18	3.85
		867	413	325	338	18	3.73
	Mean ± S.E.		428 ± 4.41	347	368 ± 7.62	19 ± 0.6	4.07 ± .12

Table 47 (Continued)

Experiment	Group	Rat no.	Body weight: Initial	Body weight: Day of repletion	Body weight: Autopsy	Food intake / day	Total nitrogen intake
			gm	gm	gm	gm	gm
II	RF10	823	460	358	472	23	8.13
		828	442	368	493	25	9.01
		833	446	364	490	24	8.49
		838	432	363	474	23	8.34
		843	414	337	411	18	6.45
		848	418	354	492	27	9.48
		853	420	332	465	26	9.44
		858	422	342	441	21	7.61
		863	422	336	476	26	9.35
		868	417	330	457	25	8.81
	Mean ± S.E.		429 ± 4.84	348	467 ± 8.10	24 ± 0.8	8.51 ± .30
	CF10	824	443	375	470	24	8.52
		829	427	362	423	19	6.98
		834	426	358			
		839	425	349	425	22	7.78
		844	415	353	439	22	7.89
		849	421	353	456	23	8.13
		854	417	321	431	24	8.57
		859	417	337			
		864	416	341	447	26	9.19
		869	415	311	417	24	8.56
	Mean ± S.E.		422 ± 3.32	346	438 ± 6.40	23 ± 0.6	8.20 ± .24

Table 47 (Continued)

Experiment	Group	Rat no.	Body weight: Initial gm	Body weight: Day of repletion gm	Body weight: Autopsy gm	Food intake day gm	Total nitrogen intake gm
II	RCE10	825	450	387	448	21	7.48
		830	439	375	469	22	7.74
		835	442	348			
		840	433	357	446	21	7.47
		845	437	359	460	22	8.02
		850	438	369			
		855	418	342	507	29	10.55
		860	418	328			
		865	416	298	491	28	10.09
		870	416	343	466	24	8.61
	Mean ± S.E.		430 ± 5.06	351	470 ± 8.42	24 ± 1.3	8.57 ± .48
	Sc	871	386		409		
		872	358		374		
		873	372		400		
		877	362		382		
		878	389				
		881	400		443		
		883	403		419		
		885	378		380		
		887	387		409		
		889	400		438		
	Mean ± S.E.		383 ± 5.57		406 ± 7.54		

Table 47 (Continued)

Experiment	Group	Rat no.	Body weight: Initial	Body weight: Day of repletion	Body weight: Autopsy	Food intake / day	Total nitrogen intake
			gm	gm	gm	gm	gm
II	Sd	874	393		412		
		875	368		385		
		876	393				
		879	354		381		
		880	368		397		
		882	403		421		
		884	390		401		
		886	378		421		
		888	376		396		
		890	374		401		
	Mean ± S.E.		378 ± 4.98		402 ± 4.73		
IIIa	C6	891	451	366	398	26	3.39
		894	451	375	389	18	2.40
		897	424	328	362	24	3.15
		900	416	340	364	18	2.29
		903	412	353	381	24	3.17
		906	434	366	408	26	3.43
		909	452	370	413	27	3.56
		912	427	335	368	24	3.17
		915	400	357	363	19	2.44
		918	442	378	400	22	2.92
	Mean ± S.E.		431 ± 5.75	357 ± 5.50	385 ± 6.22	23 ± .12	2.99 ± .02

Table 47 (Continued)

Experiment	Group	Rat no.	Body weight: Initial gm	Body weight: Day of repletion gm	Body weight: Autopsy gm	Food intake / day gm	Total nitrogen intake gm
IIIa	CF10	892	449	369	447	25	5.45
		895	451	367	450	26	5.48
		898	434	372	456	24	5.22
		901	417	352	419	25	5.40
		904	436	355	400	22	4.62
		907	410	337	423	26	5.58
		910	434	358	418	24	5.23
		913	431	342	417	24	5.13
		916	407	338	422	24	5.06
		919	434	359	420	21	4.59
	Mean ± S.E.		430 ± 4.69	355 ± 4.01	427 ± 5.61	24 ± .45	5.18 ± .11
	RCE10	893	443	360	437	22	4.88
		896	450	359	457	26	5.66
		899	438	347	433	24	5.39
		902	409	342	423	25	5.55
		905	433	351	450	24	5.36
		908	413	335	435	26	5.85
		911	433	356	454	26	5.70
		914	434	355	432	22	4.93
		917	413	356	452	26	5.75
		920	445	378	493	28	6.24
	Mean ± S.E.		431 ± 4.59	354 ± 3.66	447 ± 6.27	25 ± .58	5.53 ± .13

Table 47 (Continued)

Experiment	Group	Rat no.	Body weight			Food intake	Total nitrogen intake
			Initial	Day of repletion	Autopsy	day	
			gm	gm	gm	gm	gm
IIIb	R6	924	446	374	431	26	3.59
		927	432	339	414	24	3.43
		930	431	351	409	24	3.37
		933	438	362	432	26	3.64
		936	439	362	417	24	3.33
		939	427	355	411	23	3.26
		942	425	357	401	22	3.13
		945	440	373	427	24	3.41
		948	414	333	377	22	3.04
		951	405	341	388	23	3.26
	Mean ± S.E.		430 ± 3.97	355 ± 4.38	411 ± 5.70	24 ± .0.42	3.35 ± .06
	RF10	925	455	366	458	27	6.08
		928	433	355	424	22	5.09
		931	428	367	460	25	5.69
		934	438	347	478	30	6.89
		937	441	374	468	24	5.41
		940	434	351	460	26	5.96
		943	435	363	462	25	5.70
		946	440	345	453	26	5.86
		949	428	337	439	25	5.77
		952	434	359	443	24	5.35
	Mean ± S.E.		437 ± 2.47	356 ± 3.64	454 ± 4.91	26 ± .67	5.78 ± .16

Table 47 (Continued)

Experiment	Group	Rat no.	Body weight: Initial gm	Body weight: Day of repletion gm	Body weight: Autopsy gm	Food intake / day gm	Total nitrogen intake gm
IIIb	Sn	926	450	386	485	25	
		929	448	348	449	24	
		932	437	370	466	22	
		935	443	353	464	27	
		938	438	344	473	25	
		941	433	363	488	27	
		944	443	368	478	24	
		947	445	368	470	23	
		950	428	360	436	22	
		953	411	351	449	24	
	Mean ± S.E.		438 ± 3.64	361 ± 3.99	466 ± 5.31	24 ± .6	
IV	R5	1	491	362	416	20	2.40
		10	487	375	429	29	2.60
		14	495	363	450	25	3.01
		26	516	410	476	26	3.08
		30	498	391	461	23	2.78
		34	500	380	418	22	2.70
		43	515	424	472	22	2.67
		47	492	381	465	25	3.05
		51	486	393	431	19	2.33
		55	483	386	428	20	2.45
		59	475	377	394	16	1.96
		68	469	360	377	17	2.05
	Mean ± S.E.		492 ± 4.06	384 ± 5.54	435 ± 8.98	22 ± 1.09	2.59 ± .11

Table 47 (Continued)

Experiment	Group	Rat no.	Body weight: Initial gm	Body weight: Day of repletion gm	Body weight: Autopsy gm	Food intake / day gm	Total nitrogen intake gm
IV	RM5	2	503	404	491	25	2.91
		6	498	362	386	16	1.86
		15	483	374	461	25	2.96
		27	524	407	497	28	3.32
		31	509	384	454	23	2.74
		35	498	399	465	23	2.74
		39	508	394	454	21	2.47
		48	505	397	487	27	3.19
		52	499	431	500	24	2.83
		56	479	376	418	18	2.17
		60	478	385	448	24	2.87
		64	470	408	455	25	2.90
	Mean ± S.E.		496 ± 4.52	393 ± 5.35	460 ± 9.60	23 ± 1.0	2.74 ± .12
	RAA6½	3	510	417	544	25	3.95
		7	494	363	498	21	3.33
		11	479	405	513	25	3.92
		28	525	402	519	28	4.30
		32	510	413	527	27	4.27
		36	500	391	476	22	3.52
		40	524	427	529	24	3.84
		44	498	405	486	24	3.68
		53	495	397	475	24	3.83
		57	482	390	489	25	3.95
		61	478	390	491	27	4.27
		65	470	400	576	29	4.58
	Mean ± S.E.		497 ± 5.15	400 ± 4.69	510 ± 6.49	25 ± .66	3.95 ± .0.1

Table 47 (Continued)

Experiment	Group	Rat no.	Body weight: Initial gm	Body weight: Day of repletion gm	Body weight: Autopsy gm	Food intake / day gm	Total nitrogen intake gm
IV	RM7½	4	505	424	535	24	4.26
		8	497	404	511	26	4.55
		12	473	371	473	25	4.33
		24	522	423	552	31	5.48
		33	497	384	524	32	5.57
		37	499	403	484	26	4.52
		41	503	410	510	23	3.40
		45	495	415	495	25	4.37
		49	501	410	508	24	4.32
		58	490	380	479	25	4.33
		62	476	398	470	21	3.67
		66	486	405	517	30	5.25
	Mean ± S.E.		495 ± 3.80	402 ± 4.77	504 ± 7.36	26 ± .94	4.50 ± .19
	RAA7½	5	495	381	500	25	4.52
		9	487	381	496	23	4.21
		13	481	373	463	22	4.02
		25	502	368	480	29	5.22
		29	509	422	491	23	4.13
		38	486	381	482	26	4.64
		42	512	396	526	23	4.12
		46	490	396	490	24	4.24
		50	496	414	483	21	3.79
		54	480	386	474	24	4.26
		63	488	384	465	23	4.18
		67	479	400	501	25	4.57
	Mean ± S.E.		492 ± 3.17	390 ± 4.64	487 ± 5.0	24 ± .60	4.32 ± .11

Table 47 (Continued)

Experiment	Group	Rat no.	Body weight: Initial gm	Body weight: Day of repletion gm	Body weight: Autopsy gm	Food intake / day gm	Total nitrogen intake gm
IV	Scol	A	484		475		
		B	481		476		
		C	482		473		
		D	484		481		
		E	488		478		
		F	480		476		
		H	488		491		
		I	474		481		
		71	489		495		
		72	492		491		
		73	494		494		
		74	490		494		
	Mean ± S.E.		486 ± 1.65		484 ± 2.46		
	DD	16	477		374		
		17	473		363		
		18	491		347		
		19	480		378		
		20	490		398		
		21	484		345		
		22	486		318		
		23	491		359		
		69	479		379		
		70	488		390		
	Mean ± S.E.		484 ± 2.01		365 ± 7.53		

Table 47 (Continued)

Experiment	Group	Rat no.	Body weight: Initial gm	Body weight: Day of repletion gm	Body weight: Autopsy gm	Food intake / day gm	Total nitrogen intake gm
IV	Sni	a	438		438		
		b	430		430		
		c	465		465		
		d	501		501		
		e	480		480		
		f	485		485		
		g	479		479		
		h	500		500		
		i	489		489		
		j	471		471		
		k	444		444		
		l	476		476		
	Mean ± S.E.		472 ± 6.72		472 ± 6.72		

Table 48. Weight, percentages of nitrogen and lipid and nucleic acid content[a] of liver - Experiments I, II, III, IV

Experiment	Group	Rat no.	Weight gm	Nitrogen %	Lipid %	RNA %	DNA %
I	Sr	81	15.22	3.54	5.5	107.2	35.7
		82	13.51	3.73	4.5	111.1	34.8
		85	11.89	3.66	4.6	92.6	34.2
		86	12.15	3.66	5.0	97.6	38.8
		89	13.68	3.50	6.6	71.2	36.8
		90	12.73	3.72	5.5	91.8	40.2
		93	11.98	3.79	5.4	96.2	35.2
		94	15.69	3.77	4.4	108.7	41.4
		97	11.55	3.68	5.3		
		98	11.22	3.80	5.8		
		101	13.60	3.43	5.2		
		102	14.76	3.62	5.1		
	Mean ± S.E.		13.16 ± .43	3.66 ± .03	5.2 ± .2	7.0 ± 4.5	37.1 ± .1
	Scly	83	13.22	3.80	5.3	86.4	31.1
		84	14.88	3.49	4.5	111.0	37.6
		87	12.49	3.69	5.2	93.4	33.7
		88	12.74	3.68	6.2	83.3	39.8
		91	16.60	3.54	5.5	96.4	45.5
		92	14.64	3.83	4.9	97.1	34.8
		95	13.95	3.75	4.7	106.6	37.2
		96	17.20	3.73	6.4	133.9	45.9
		99	17.10	3.44	4.3		
		100	14.90	3.88	4.7		
		103	13.75	3.80	5.0		
		104	14.50	3.74	4.7		
	Mean ± S.E.		14.66 ± .46	3.70 ± .04	5.1 ± .2	101.0 ± 5.7	38.2 ± 1.8

[a]No analysis for Experiment II.

Table 48 (Continued)

Experiment	Group	Rat no.	Weight gm	Nitrogen %	Lipid %	RNA %	DNA %
I	Scol	117	10.01	3.89	4.0	73.7	27.0
		118	10.68	3.86	4.8	82.4	30.7
		119	11.84	3.66	4.3	81.9	34.7
		120	12.93	3.46	4.1	88.8	35.3
		121	12.21	3.61	5.0	93.4	40.9
		122	12.55	3.58	4.7	98.7	35.4
		123	13.76	3.58	3.1	99.3	34.0
		124	11.59	3.82	5.6	106.0	31.6
		125	12.10	3.71	4.4[b]		
		126	13.20	3.73	4.8		
		127	11.45	3.53	4.1		
		128	10.60	3.97	4.3		
Mean ± S.E.			11.91 ± .32	3.70 ± .04	4.4 ± .2	90.5 ± 3.83	3.7 ± 1.42
	DD	105	8.72	2.78	7.3		
		106	10.65	2.31	8.6		
		107	9.52	2.60	7.7		
		108	10.73	2.48	7.4		
		109	11.59	2.22	4.4		
		110	8.90	2.97	8.1		
		111	9.22	2.74	6.7		
		112	9.62	2.59	6.2		
		113	9.00	2.82	4.7		
		114	10.55	2.66	9.9		
		115	11.83	2.40	14.1		
		116	9.06	2.92	4.6		
Mean ± S.E.			9.95 ± .31	2.62 ± .07	7.5 ± .8		

[b]Sample lost after N was determined. Average of 11.

Table 48 (Continued)

Experiment	Group	Rat no.	Weight gm	Nitrogen %	Lipid %	RNA %	DNA %
II	R6	821					
		826	15.60	2.01	11.0		
		831					
		836	11.20	2.55	8.8		
		841	11.95	2.43	6.7		
		846	12.30	2.58	6.7		
		851	13.85	2.54	11.4		
		856	11.10	2.80	10.3		
		861	15.55	2.25	13.3		
		866	12.70	2.48	8.8		
Mean ± S.E.			13.03 ± .63	2.46 ± .08	9.6 ± .1		
	C6	822	10.40	2.54	5.9		
		827					
		832	9.60	2.85	5.8		
		837	11.00	2.56	8.2		
		842	11.00	2.64	4.4		
		847	11.65	2.55	10.8		
		852	11.90	2.64	5.4		
		857	12.40	2.30	11.8		
		862	11.80	2.72	4.9		
		867	8.80	2.88	5.8		
Mean ± S.E.			10.95 ± .39	2.62 ± .06	7.0 ± .9		

Table 48 (Continued)

Experiment	Group	Rat no.	Weight gm	Nitrogen %	Lipid %	RNA %	DNA %
II	RF10	823	13.80	2.82	4.1		
		828	15.4	2.74	5.2		
		833	14.85	2.92	6.4		
		838	12.70	2.94	6.4		
		843	10.95	3.01	5.0		
		848	14.65	2.90	5.7		
		853	12.15	3.30	4.5		
		858	13.25	2.95	4.7		
		863	14.45	2.93	6.1		
		868	14.90	2.94	5.6		
Mean ± S.E.			13.71 ± .45	2.94 ± .05	5.4 ± .3		
	CF10	824	12.60	2.80	5.2		
		829	12.30	2.92	4.3		
		834					
		839	10.95	2.94	5.6		
		844	11.70	3.02	5.8		
		849	13.80	3.11	7.3		
		854	11.60	3.10	5.2		
		859					
		864	14.40	2.78	4.0		
		869	14.00	2.87	4.4		
Mean ± S.E.			12.67 ± .45	2.94 ± .04	5.2 ± .1		

Table 48 (Continued)

Experiment	Group	Rat no.	Weight gm	Nitrogen %	Lipid %	RNA %	DNA %
II	RCE10	825	13.90	2.20	4.6		
		830	12.65	3.11	4.9		
		835					
		840	14.05	2.71	3.3		
		845	12.65	3.31	4.9		
		850					
		855	15.70	3.09	6.0		
		860					
		865	17.20	2.91	5.7		
		870	15.50	3.34	3.7		
Mean ± S.E.			14.52 ± .64	2.95 ± .15	4.7 ± .4		
	Sc	871	15.70	3.12	3.7		
		872	14.10	2.96	3.8		
		873	15.95	3.53	4.2		
		877	12.90	3.52	4.2		
		878					
		881	14.20	3.05	4.6		
		883	13.70	3.43	4.2		
		885	12.95	3.86	4.0		
		887	13.70	3.38	3.7		
		889	17.50	3.19	3.8		
Mean ± S.E.			14.52 ± .51	3.34 ± .10	4.0 ± .1		

Table 48 (Continued)

Experiment	Group	Rat no.	Weight gm	Nitrogen %	Lipid %	RNA %	DNA %
II	Sd	874	13.65	3.54	4.2		
		875	11.20	3.39	3.8		
		876					
		879	11.35	3.39	4.1		
		880	13.20	3.42	4.0		
		882	14.70	2.81	3.7		
		884	15.40	2.75	3.8		
		886	16.50	3.33	3.9		
		888	13.80	3.42	4.1		
		890	15.70	3.03	5.9		
Mean ± S.E.			14.00 ± .59	3.23 ± .10	4.2 ± 0.2		
IIIa	C6	891	9.15	3.19	5.3	80.2	34.8
		894	9.05	3.24	11.5	58.7	34.8
		897	9.00	2.78	6.5	66.0	31.6
		900	10.10	2.69	8.6	70.6	35.0
		903	9.20	3.08	8.7	78.9	31.8
		906	9.55	3.04	4.6	75.2	39.8
		909	11.10	2.66	13.5	70.2	26.8
		912	9.25	2.98	7.7	72.2	23.7
		915	8.20	2.94	11.7	61.2	27.3
		918	11.05	4.10	14.5	67.4	36.9
Mean ± S.E.			9.56 ± .29	3.07 ± .13	9.3 ± 1.1	70.1 ± 2.23	32.2 ± 1.59

Table 48 (Continued)

Experiment	Group	Rat no.	Weight gm	Nitrogen %	Lipid %	RNA %	DNA %
IIIa	CF10	892	13.20	2.81	6.4	84.8	26.0
		895	12.90	3.22	4.7	85.3	28.7
		898	12.30	2.90	6.7	93.8	33.8
		901	10.30	3.45	5.2	82.7	37.0
		904	11.35	3.20	5.6	78.7	34.6
		907	12.20	3.36	4.6	90.2	31.6
		910	12.25	3.23	7.5	86.1	30.2
		913	10.35	3.25	5.7	79.8	29.2
		916	11.20	3.13	5.4	75.3	32.4
		919	10.00	3.44	5.3	81.1	36.1
Mean ± S.E.			11.61 ± .36	3.20 ± .02	5.7 ± .3	83.8 ± 1.73	32.0 ± 1.11
	RCE10	893	11.60	3.23	4.1	84.5	37.1
		896	12.20	3.24	5.4	92.8	24.8
		899	12.90	2.82	5.1	81.0	39.4
		902	11.15	3.36	5.5	71.4	36.1
		905	12.50	3.12	5.0	84.5	38.8
		908	16.00	3.08	4.0	121.2	42.3
		911	12.90	2.95	4.3	95.4	27.2
		914	11.00	3.09	4.5	77.6	32.1
		917	13.50	3.33	5.4	67.2	36.1
		920	16.50	2.68	9.5	120.7	40.3
Mean ± S.E.			13.02 ± .59	3.09 ± .02	5.3 ± .5	89.6 ± 5.88	35.4 ± 1.80

Table 48 (Continued)

Experiment	Group	Rat no.	Weight gm	Nitrogen %	Lipid %	RNA %	DNA %
IIIb	R6	924	12.90	2.50	8.7	75.5	26.0
		927	10.25	2.95	6.4	76.0	21.9
		930	9.75	2.74	8.9	80.0	23.8
		933	13.80	2.34	10.9	78.4	30.9
		936	15.50	1.85	22.7	84.6	29.1
		939	11.60	2.39	9.0	73.9	24.4
		942	14.35	2.03	11.7	81.9	28.8
		945	14.50	2.20	15.9	84.8	25.4
		948	10.10	2.57	10.8	73.4	23.2
		951	17.50	1.90	17.0	91.3	28.5
Mean ± S.E.			13.02 ± .81	2.35 ± .11	12.20± 1.6	80.0 ± 1.80	26.2 ± .94
	RF10	925	12.00	3.60	6.1	83.2	28.6
		928	11.10	2.84	5.3	68.2	23.5
		931	11.75	3.19	4.8	77.8	25.2
		934	16.10	3.10	5.5	120.1	33.2
		937	13.10	3.25	6.2	103.2	32.4
		940	13.65	3.21	4.4	102.1	27.1
		943	14.60	2.87	6.5	95.0	28.4
		946	11.80	3.06	4.7	104.2	28.6
		949	13.60	3.07	4.4	106.1	27.8
		952	12.25	3.02	5.8	93.0	26.3
Mean ± S.E.			13.00 ± .48	3.12 ± .07	5.4 ± 0.2	95.3 ± 4.84	28.1 ± .94

Table 48 (Continued)

Experiment	Group	Rat no.	Weight gm	Nitrogen %	Lipid %	RNA %	DNA %
IIIb	Sr	926	12.20	3.60	4.4		
		929	11.90	3.12	5.0		
		932	12.00	3.14	5.1		
		935	12.25	3.60	5.0		
		938	11.80	3.57	3.9		
		941	14.05	3.54	4.0		
		944	13.10	3.56	5.0		
		947	14.00	3.28	4.4		
		950	11.40	3.51	4.9		
		953	11.80	3.42	5.6		
Mean ± S.E.			12.45 ± .30	3.43 ± .06	4.7 ± 0.2		
IV	R5	1	11.05	2.46	7.5	70.4	18.5
		10	11.41	2.61	13.4	78.9	28.4
		14	17.40	1.92	31.1	97.4	34.0
		26	14.30	2.43	16.1	91.0	27.5
		30	11.80	2.53	12.5	86.2	23.8
		34	8.09	3.51	9.3	83.9	26.4
		43	17.04	1.90	20.9	84.0	29.0
		47	13.49	2.50	11.9	90.1	28.0
		51	10.00	2.87	18.0	69.1	36.8
		55	9.16	2.87	19.1	76.6	40.3
		59	8.50	3.17	6.9	67.3	28.4
		68	8.50	2.96	8.6	94.2	34.4
Mean ± S.E.			11.73 ± .93	2.64 ± .14	14.6 ± 2.0	80.8 ± 2.78	29.6 ± 1.72

Table 48 (Continued)

Experiment	Group	Rat no.	Weight gm	Nitrogen %	Lipid %	RNA %	DNA %
IV	RM5	2	13.28	2.87	8.1	101.1	29.9
		6	12.20	2.76	11.2	104.4	41.0
		15	10.70	2.88	8.8	87.0	30.0
		27	13.60	2.63	14.6	77.0	19.6
		31	14.32	2.54	8.8	91.5	27.1
		35	12.18	2.69	16.6	89.4	26.0
		39	11.02	2.40	6.5	73.0	21.3
		48	12.96	2.48	11.2	87.0	25.3
		52	13.63	2.52	15.7	87.0	33.5
		56	8.90	2.93	7.7	71.8	29.4
		60	10.60	2.88	9.7	79.9	42.6
		64	11.48	2.80	15.4	78.8	40.9
Mean ± S.E.			12.07 ± .46	2.70 ± .05	11.2 ± 1.0	85.7 ± 2.95	30.6 ± 2.19
	RAA6½	3	18.39	2.34	21.2	129.3	35.1
		7	13.73	3.03	8.6	110.7	35.6
		11	16.07	2.54	16.1	106.2	34.6
		28	15.28	2.61	12.4	98.5	25.0
		32	14.68	2.52	8.2	102.8	31.1
		36	11.17	3.05	6.6	90.2	25.4
		40	15.70	2.68	8.4	104.6	26.7
		44	12.24	2.99	11.2	96.6	28.7
		53	12.30	2.88	10.6	97.3	34.7
		57	12.60	2.58	6.3	83.3	34.2
		61	12.50	2.98	7.6	88.1	43.1
		65	15.70	2.63	17.3	107.8	47.7
Mean ± S.E.			14.20 ± .61	2.74 ± .07	11.21± 1.4	101.3 ± 3.51	33.5 ± 1.98

Table 48 (Continued)

Experiment	Group	Rat no.	Weight gm	Nitrogen %	Lipid %	RNA %	DNA %
1V	RM7½	4	16.10	2.59	19.8	113.5	29.1
		8	13.81	2.92	7.1	104.6	36.7
		12	10.99	2.97	10.1	85.8	27.1
		24	13.50	2.85	9.2	106.8	24.8
		33	15.61	2.38	16.6	101.2	26.6
		37	12.06	2.63	12.4	96.5	29.2
		41	16.39	2.34	9.2	120.8	29.4
		45	11.40	3.24	7.7	92.4	27.7
		49	12.04	3.28	9.1	94.6	38.6
		58	11.00	2.86	11.3	81.4	30.6
		62	10.30	3.15	10.8	78.0	40.4
		66	12.50	3.12	12.2	99.1	38.8
Mean ± S.E.			12.98 ± .61	2.86 ± .09	11.29 ± 1.1	97.9 ± 3.65	31.6 ± 1.58
	RAA7½	5	12.39	3.08	6.0	93.2	21.4
		9	12.50	2.95	5.2	93.5	30.4
		13	11.97	2.46	6.8	80.8	26.3
		25	11.58	3.39	8.4	72.2	22.4
		29	11.19	3.06	6.7	89.9	26.9
		38	13.70	2.87	11.0	98.8	26.9
		42	15.65	3.02	6.7	121.6	25.8
		46	12.62	3.17	5.6	97.2	27.6
		50	11.32	3.12	9.9	87.2	35.8
		54	11.12	3.21	6.3	78.7	32.2
		63	12.65	2.92	10.9	96.6	42.2
		67	11.25	3.09	8.6	81.0	37.5
Mean ± S.E.			12.33 ± .39	3.03 ± .07	7.76 ± 0.6	90.9 ± 3.68	29.6 ± 1.80

Table 48 (Continued)

Experiment	Group	Rat no.	Weight gm	Nitrogen %	Lipid %	RNA %	DNA %
IV	Scol	A	11.60	3.65	5.6	87.6	22.3
		B	14.19	3.63	6.4	117.0	36.1
		C	12.71	3.49	4.7	102.2	34.8
		D	13.41	3.58	6.0	92.6	26.1
		E	13.52	3.71	6.0	110.5	29.1
		F	10.51	3.80	4.9	90.5	21.8
		H	12.99	3.81	5.7	107.3	22.2
		I	14.00	3.72	5.7	105.0	33.8
		71	11.56	3.63	6.1	87.5	31.2
		72	11.61	3.96	4.9		
		73	11.84	4.00	4.8		
		74	10.13	3.98	6.1		
Mean ± S.E.			12.34 ± .38	3.75 ± .05	5.6 ± .2	100 ± 1.14	28.6 ± 1.90
	DD	16	12.11	2.16	14.3	77.1	23.8
		17	8.95	2.66	7.6	61.8	29.2
		18	9.75	2.21	9.6	65.6	18.6
		19	11.09	2.21	12.9	69.3	23.8
		20	10.10	2.49	7.3	63.7	24.4
		21	8.82	2.47	12.6	59.0	24.9
		22	9.11	2.51	7.6	62.4	19.6
		23	8.92	2.65	6.4	65.0	22.4
		69	9.50	2.24	6.9	57.4	36.1
		70	8.70	2.60	7.1	66.5	32.7
Mean ± S.E.			9.70 ± .35	2.42 ± .06	9.2 ± .9	65. ± .18	25.6 ± 1.74

Table 48 (Continued)

Experiment	Group	Rat no.	Weight gm	Nitrogen %	Lipid %	RNA %	DNA %
IV	Sni	a	12.45	3.67	4.5	99.4	20.8
		b	9.81	3.42	6.3	84.1	20.2
		c	15.08	3.24	4.7	119.0	41.7
		d	13.25	3.30	5.2	75.8	21.5
		e	12.05	3.76	5.7	101.0	24.8
		f	11.84	3.34	5.1	97.5	21.3
		g	11.48	3.46	4.6	88.6	22.1
		h	12.62	3.70	5.8	101.0	25.4
		i	12.56	3.59	5.7	93.3	34.8
		j	11.91	3.68	4.9	103.4	38.8
		k	10.60	3.71	5.8	73.3	22.2
		l	11.90	3.62	4.5	82.4	25.9
Mean ± S.E.			12.13 ± .38	3.54 ± .05	5.2 ± .18	93.2 ± 3.76	26.6 ± 2.15

Table 49. Spleen weights and agglutinins of each rat - Experiment II

Group	Rat no.	Spleen weight	6th day log titer	10th day log titer	14th day log titer
R6	821				
	826	0.70	2.11	2.11	1.65
	831				
	836	0.80	2.56	2.41	1.65
	841	0.55	1.96	1.50	1.20
	846	0.60	2.11	2.11	1.50
	851	0.85	2.11	1.65	1.35
	856	1.10	1.96	1.81	1.81
	861	0.90	1.50	0.90	0.75
	866	0.60	2.11	1.65	1.50
Mean ± S.E.		.76 ± .07	2.05 ± .10	1.78 ± .17	1.43 ± .12
C6	822	0.50	1.35	1.20	1.05
	827				
	832	0.65	1.81	1.65	1.35
	837	0.55	1.65	2.11	2.11
	842	0.70	2.41	1.81	1.20
	847	0.75	2.56	2.26	2.26
	852	0.65	2.11	1.65	1.35
	857	0.65	2.11	1.81	1.50
	862	0.80	2.11	2.11	1.65
	867	0.70	2.56	1.81	1.81
Mean ± S.E.		.66 ± .10	2.07 ± .14	1.82 ± .1	1.59 ± .14

Table 49 (Continued)

Group	Rat no.	Spleen weight	6th day log titer	10th day log titer	14th day log titer
RF10	823	0.70	2.41	2.41	2.26
	828	0.80	2.26	1.81	2.26
	833	1.10	2.11	1.81	1.81
	838	0.85	2.11	2.11	2.11
	843	0.95	2.56	2.56	2.26
	848	0.85	2.71	2.11	2.11
	853	0.85	1.50	1.50	1.35
	858	1.05	2.41	2.41	2.11
	863	1.45	2.11	2.11	2.11
	868	.80	0.95	0.30	0.60
Mean ± S.E.		.94 ± .07	2.11 ± .17	1.91 ± .21	1.90 ± .17
CF10	824	1.30	2.71	2.71	2.26
	829	0.90	2.41	1.81	2.41
	834				
	839	0.75	2.86	1.96	1.96
	844	0.85	2.56	2.11	1.65
	849	0.75	1.81	1.81	1.35
	854	0.80	1.65	1.50	1.50
	859				
	864	0.90	2.41	2.26	2.11
	869		2.71	2.11	1.81
Mean ± S.E.		.89 ± .13	2.39 ± .16	2.03 ± .13	1.88 ± .13

Table 49 (Continued)

Group	Rat no.	Spleen weight	6th day log titer	10th day log titer	14th day log titer
RCE10	825	0.70	1.81	1.81	1.81
	830	0.95	1.96	1.65	1.65
	835				
	840	1.15	2.71	2.11	1.65
	845	0.85	0	0.30	0.60
	850				
	855	1.00	1.65	1.65	1.50
	860				
	865	1.20	2.41	2.11	1.20
	870	0.90	2.26	1.96	1.35
Mean ± S.E.		.96 ± .06	1.83 ± .34	1.65 ± .24	1.39 ± .15
Sc	871	0.80	2.11	2.11	1.96
	872	1.00	2.56	2.11	2.11
	873	1.00	1.65	1.50	1.05
	877	0.65	2.26	2.26	1.65
	878				
	881	1.10	2.71	1.65	1.81
	883	1.10	1.50	1.50	1.35
	885	1.00	0.30	0.75	1.20
	887	0.80	2.26	1.96	1.96
	889	1.00	2.56	2.11	2.11
Mean ± S.E.		.94 ± .05	1.99 ± .25	1.77 ± .16	1.69 ± .13

Table 49 (Continued)

Group	Rat no.	Spleen weight	6th day log titer	10th day log titer	14th day log titer
Sd	874	1.15	1.65	1.50	1.35
	875	0.70	1.96	1.81	1.81
	876				
	879	0.85	2.86	2.71	2.26
	880	0.85	1.96	1.50	0.90
	882	0.70	1.81	1.81	1.81
	884	0.95	2.71	1.96	1.35
	886	1.05	2.56	2.56	1.96
	888	0.60	2.41	2.11	1.81
	890	1.00	2.41	2.26	2.11
Mean ± S.E.		.87 ± .02	2.26 ± .14	2.02 ± .14	1.71 ± .14

Table 50. Spleen weights, circulating antibody titers, spleen cell and plaque-forming cell counts of each rat - Experiment III

Group	Rat no.	Weight	Spleen		Circulating antibodies	
			Total cell count	Plaque-forming cell	Agglutinin	Hemolysin
		gm	x 10^7	log	log titer	log titer
C6	891	0.60	135	2.18	1.05	3.35
	894	0.70	165	2.55	1.05	3.58
	897	0.50	101	1.66	1.05	2.90
	900	0.60	62	2.25	1.35	3.35
	903	0.50	104	2.49	1.35	3.81
	906	0.50	122	2.39	1.20	2.97
	909	0.40	45	2.28	0.90	2.30
	912	0.50	83	2.62	0.48	2.00
	915	0.60	103	2.26	0.30	1.42
	918	0.60	184	3.01	0.90	3.73
Mean ± S.E.		.55 ± .03	110 ± 14	2.37 ± .11	.96 ± .11	2.94 ± .25
CF10	892	0.60	107	2.08	1.05	3.20
	895	0.70	119	2.26	1.05	3.35
	898	0.85	127	2.46	1.35	3.58
	901	0.75	182	2.05	0.75	2.45
	904	0.70	167	2.74	1.05	3.28
	907	0.60	140	2.83	1.35	3.35
	910	0.85	245	3.17	1.20	2.45
	913	0.50	98	2.76	1.35	3.73
	916	0.90	203	2.73	0.90	2.75
	919	0.62	218	3.40	0.61	3.05
Mean ± S.E.		.71 ± .04	16 ± 16	2.65 ± .14	1.07 ± .08	3.12 ± .14

Table 50 (Continued)

Group	Rat no.	Weight	Spleen		Circulating antibodies	
			Total cell count	Plaque-forming cell	Agglutinin	Hemolysin
		gm	x 10^7	log	log titer	log titer
RCE10	893	0.82	119	2.51	1.35	3.20
	896	0.85	202	2.64	1.05	3.28
	899	0.75	118	2.65	0.75	2.83
	902	0.60	88	2.26	0.75	2.15
	905	0.70	102	2.40	1.20	3.66
	908	0.90	173	2.93	1.05	2.45
	911	0.70	241	3.29	0.90	2.00
	914	0.70	104	2.72	1.35	3.96
	917	0.72	80	2.68	1.05	3.05
	920	0.85	206	3.36	1.05	3.05
Mean ± S.E.		.76 ± .01	143 ± 18	2.74 ± .11	1.05 ± .02	2.96 ± .20
R6	924	0.50	27		0.90	3.20
	927	0.55	99	1.56	0.60	2.45
	930	0.65	86	2.38	1.05	3.35
	933	0.60	50		1.05	3.28
	936	0.70	140	1.50	0.90	2.90
	939	0.50	65	0.90	0.90	2.30
	942	0.70	82	2.56	1.65	3.66
	945	0.52	92	1.42	0.38	3.05
	948	0.50	51	1.00	0.75	2.30
	951	0.72	123	2.63	1.20	3.35
Mean ± S.E.		.59 ± .03	81 ± 11	1.74 ± .24	.94 ± .11	2.99 ± .16

Table 50 (Continued)

Group	Rat no.	Weight	Spleen		Circulating antibodies	
			Total cell count	Plaque-forming cell	Agglutinin	Hemolysin
		gm	x 10^7	log	log titer	log titer
RF10	925	0.80	114		1.50	3.66
	928	0.60	91	1.42	0.30	2.00
	931	0.60	77	2.33	1.05	2.30
	934	0.80	88		1.20	3.12
	937	1.00	370	3.09	2.11	3.66
	940	0.62	166	2.52	1.35	3.35
	943	0.90	116	2.39	0.90	2.90
	946	0.80	96	2.42	1.65	3.50
	949	0.65	122	1.88	0.60	2.30
	952	0.85	178	2.74	1.20	2.75
Mean ± S.E.		.76 ± .04	142 ± 27	2.35 ± .18	1.19 ± .16	2.95 ± .19
Sr	926	0.71	156		0.60	2.68
	929	0.90	205	2.59	1.50	3.50
	932	0.70	134	1.97	1.20	3.20
	935	0.70	233		0.90	2.22
	938	0.70	106	1.43	0.60	2.09
	941	0.80	153	0	0	2.00
	944	1.00	188	2.83	1.50	3.58
	947	0.70	113	1.04	1.05	2.98
	950	0.60	98	2.23	1.20	2.75
	953	0.85	149	2.65	1.35	2.90
Mean ± S.E.		.76 ± .04	153 ± 14	1.84 ± .34	.99 ± .15	2.79 ± .18

Table 51. Spleen weights, spleen cell and plaque-forming cell counts and percentage nucleated cells, and circulating antibody titers of each rat - Experiments I and IV

Rat no.	Weight	Spleen			Circulating antibodies	
		Total cell count	Nucleated cell	Plaque-forming cell	Agglutinin	Hemolysin
	gm	x 10^7	%	log	log titer	log titer
Experiment I Group Sr						
81	0.88	205	53.1	1.51	1.65	2.65
82	0.80	136	27.2	2.23	2.16	3.55
85	0.61	100	37.1	1.61	1.56	3.25
86	0.92	125	44.5	1.73	1.05	2.41
89	0.95	207	44.5	2.89	2.56	3.79
90	0.75	138	40.7	2.65	1.86	3.91
93	1.07	175	40.6	1.43	1.38	2.56
94	0.86	122	36.7	1.57	1.35	2.86
97	0.88	226	27.4	1.34	1.05	2.86
98	1.24	290	23.1	2.85	2.56	3.91
101	0.82	154	25.4	1.15	1.96	3.02
102	0.84	162	28.0	2.57	2.26	4.01
Mean ± S.E.						
	.88 ± .05	170 ± 15	35.6 ± 2.71	1.96 ± .18	1.78 ± .15	3.23 ± .17

Table 51 (Continued)

Rat no.	Weight	Spleen			Circulating antibodies	
		Total cell count	Nucleated cell	Plaque-forming cell	Agglutinin	Hemolysin
	gm	x 10^7	%	log	log titer	log titer
Experiment I						
Group Scly						
83	1.05	191	32.6	2.57	2.16	3.49
84	1.13	190	30.3	2.25	1.96	3.91
87	0.88	201	31.8	1.60	1.35	2.56
88	0.98	185	37.1	2.48	2.35	3.41
91	1.06	157	34.4	2.36	2.46	3.79
92	1.09	207	35.5	2.66	2.46	3.79
95	0.83	174	46.7	2.18	1.26	2.56
96	1.01	198	28.9	2.57	1.96	3.19
99	1.15	194	43.1	1.30	1.05	2.77
100	0.90	123	37.6	2.07	1.56	3.31
103	1.05	168	29.5	2.05	1.05	2.86
104	0.80	95	37.2	1.40	0.75	0.45
Mean ± S.E.						
	.99 ± .01	174 ± 10	35.4 ± 1.54	2.12 ± .13	1.70 ± .18	3.01 ± .27

Table 51 (Continued)

Rat no.	Weight	Spleen			Circulating antibodies	
		Total cell count	Nucleated cell	Plaque-forming cell	Agglutinin	Hemolysin
	gm	x 10^7	%	log	log titer	log titer
Experiment I						
Group Scol						
117	1.05				2.20	4.01
118	0.80				2.20	4.09
119	1.20				2.50	3.68
120	0.97				1.96	2.86
121	1.00				2.16	3.49
122	0.83				0.95	2.71
123	1.02				1.96	2.70
124	0.90				2.26	3.86
125	1.32				0.45	0
126	0.82				2.16	3.91
127	0.90				0.95	2.26
128	0.89				2.46	4.09
Mean ± S.E.						
	.93 ± .04				1.85 ± .19	3.14 ± .33

Table 51 (Continued)

Rat no.	Weight	Spleen			Circulating antibodies	
		Total cell count	Nucleated cell	Plaque-forming cell	Agglutinin	Hemolysin
	gm	x 10^7	%	log	log titer	log titer
Experiment I						
Group DD						
105	0.59				2.46	3.86
106	0.80				2.26	3.38
107	0.44				2.16	3.49
108	0.62				0.45	1.90
109	0.73				2.46	3.49
110	0.59				1.65	2.56
111	0.58				1.86	3.19
112	0.65				2.46	3.71
113	0.50				0.95	2.56
114	0.75				2.56	3.75
115	0.50				0.45	1.05
116	0.91				1.26	2.81
Mean ± S.E.						
	.64 ± .04				1.75 ± .23	2.98 ± .24

Table 51 (Continued)

Rat no.	Weight	Spleen			Circulating antibodies	
		Total cell count	Nucleated cell	Plaque-forming cell	Agglutinin	Hemolysin
	gm	x 10^7	%	log	log titer	log titer
Experiment IV						
Group R5						
1	0.40	45	33.9	1.36	1.26	1.34
10	0.48	38	43.7	1.92	1.65	2.46
14	0.50	42	60.8	0.60	0.75	1.47
26	0.65	68	56.7	1.45	0.38	1.11
30	0.63	93	25.1	1.43	1.26	1.96
34	0.50	23	52.9	0.85	0.45	0.95
43	0.59	51	54.5	1.00	0.38	0.80
47	0.50	41	78.5	1.35	0.30	0.95
51	0.46	48	57.2	1.30	1.05	1.26
55	0.40	39	60.6	0.90	0.65	0.90
59	0.40	28	85.0	2.36	1.96	2.46
68	0.61	110	72.2	2.16	1.86	2.02
Mean ± S.E.						
	.51 ± .03	52 ± 7	56.8 ± 4.96	1.39 ± .15	1.00 ± .17	1.48 ± .17

Table 51 (Continued)

Rat no.	Weight	Spleen			Circulating antibodies	
		Total cell count	Nucleated cell	Plaque-forming cell	Agglutinin	Hemolysin
	gm	x 10^7	%	log	log titer	log titer
Experiment IV						
Group RM5						
2	0.67	83	40.7	0.90	0.75	1.11
6	0.68	95	56.0	2.72	1.35	2.15
15	0.63	57	89.6	2.26	1.26	1.54
27	0.48	49	36.6	1.53	0.38	0.95
31	0.53	51	54.4	2.09	1.96	1.90
35	0.50	27	63.7	0.90	0.95	1.04
39	0.57	64	33.6	0.75	1.26	1.86
48	0.57	37	49.6	0.90	0.65	1.15
52	0.73	70	53.7	2.24	1.35	1.26
56	0.47	33	70.1	0.75	0.60	1.26
60	0.50	58	66.8	2.59	1.96	2.02
64	0.55	54	67.7	2.23	1.96	2.33
Mean ± S.E.						
	.57 ± .02	57 ± 6	56.9 ± 4.60	1.66 ± .23	1.20 ± .16	1.55 ± .14

Table 51 (Continued)

Rat no.	Weight	Spleen			Circulating antibodies	
		Total cell count	Nucleated cell	Plaque-forming cell	Agglutinin	Hemolysin
	gm	x 10^7	%	log	log titer	log titer
Experiment IV Group RAA6½						
3	0.62	45	39.8	1.11	1.26	1.42
7	0.70	88	50.8	1.23	0.65	0.95
11	0.82	128	53.6	2.12	1.05	1.48
28	0.71	55	77.1	1.66	0.65	1.53
32	0.72	100	54.6	1.30	1.05	1.72
36	0.75	122	68.2	1.56	0.38	1.26
40	0.53	52	48.4	1.76	0.95	1.26
44	0.65	77	47.6	1.66	0.95	1.11
53	0.50	62	49.1	2.18	1.96	2.32
57	0.65	56	61.7	2.19	2.56	2.46
61	0.50	55	45.7	2.70	2.56	2.76
65	0.60	66	76.5	2.83	1.86	2.46
Mean ± S.E.						
	.65 ± .03	76 ± 8	56.1 ± 3.5	1.86 ± .16	1.32 ± .21	1.73 ± .02

Table 51 (Continued)

Rat no.	Weight	Spleen			Circulating antibodies	
		Total cell count	Nucleated cell	Plaque-forming cell	Agglutinin	Hemolysin
	gm	x 10^7	%	log	log titer	log titer
Experiment IV Group RM7½						
4	0.68	47	73.3	1.77	1.86	1.78
8	0.55	39	57.3	2.11	1.56	2.20
12	0.52	35	45.3	1.04	1.56	2.00
24	0.75	74	64.6	1.18	0.65	1.75
33	0.65	70	53.2	2.47	1.86	2.02
37	0.70	65	46.2	2.80	2.56	3.31
41	0.64	55	53.3	1.20	0.65	0.95
45	0.54	37	61.1	1.43	0.18	0.95
49	0.66	64	72.4	2.65	1.96	2.32
58	0.66	51	63.7	2.31	2.56	2.62
62	0.51	42	60.2	2.60	1.96	2.92
66	0.70	72	39.8	2.30	1.86	1.56
Mean ± S.E.	.63 ± .02	54 ± 4	57.5 ± 3.02	1.99 ± .18	1.60 ± .22	2.03 ± .21

Table 51 (Continued)

Rat no.	Weight	Spleen			Circulating antibodies	
		Total cell count	Nucleated cell	Plaque-forming cell	Agglutinin	Hemolysin
	gm	x 10^7	%	log	log titer	log titer
Experiment IV						
Group RAA7½						
5	0.60	38	53.3	0.90	0.65	1.42
9	0.81	68	56.2	1.97	2.16	2.98
13	0.45	24	37.4	2.21	1.65	1.60
25	0.65	93	57.6	2.80	1.96	2.15
29	0.60	84	22.1	0.90	1.56	1.26
38	0.84	99	55.6	2.45	1.96	1.81
42	0.72	87	41.4	2.84	1.96	2.32
46	0.59	50	79.8	2.64	1.65	2.55
50	0.48	44	40.4	1.93	1.26	1.96
54	0.51	50	38.9	1.57	1.96	2.49
63	0.63	82	80.9	2.94	1.86	2.03
67	0.80	87	83.1	2.88	2.56	2.70
Mean ± S.E.						
	.64 ± .04	67 ± 7	53.9 ± 5.68	2.17 ± .21	1.76 ± .14	2.11 ± .15

Table 51 (Continued)

Rat no.	Weight	Spleen			Circulating antibodies	
		Total cell count	Nucleated cell	Plaque-forming cell	Agglutinin	Hemolysin
	gm	x 10^7	%	log	log titer	log titer
Experiment IV						
Group Scol						
A	0.79	82	32.4	2.09	1.56	1.90
B	1.05	313	34.1	2.73	1.86	1.90
C	0.76	222	28.8	2.35	1.86	2.20
D	0.89	129	32.5	1.97	1.56	1.60
E	0.60	113	63.5	1.75	0.38	1.20
F	0.50	240	46.6	1.90	1.56	1.54
H	0.68	120	47.2	1.79	1.05	1.42
I	0.69	71	71.2	2.86	2.26	2.46
71	0.70	170	51.5	3.06	1.96	1.86
72	0.48	63	51.2	1.81	1.35	1.72
73	0.50	90	55.2	1.95	0.90	1.42
74	0.74	141	62.6	2.89	2.16	2.55
Mean ± S.E.						
	.70 ± .05	146 ± 22	48.1 ± 4.00	2.26 ± .14	1.54 ± .16	1.81 ± .12

Table 51 (Continued)

Rat no.	Weight	Spleen			Circulating antibodies	
		Total cell count	Nucleated cell	Plaque-forming cell	Agglutinin	Hemolysin
	gm	x 10^7	%	log	log titer	log titer
Experiment IV Group DD						
16	0.45	63	26.4	1.86	1.26	1.90
17	0.67	57	42.9	1.35	1.35	2.20
18	0.52	76	49.3	1.36	1.05	1.54
19	0.42	42	19.0	0.95	0.65	1.11
20	0.50	43	29.6	0.95	0.75	1.34
21	0.55	64	21.8	1.83	1.35	1.42
22	0.43	55	37.2	1.55	1.35	2.20
23	0.48	90	22.2	2.12	1.65	2.20
69	0.40	45	33.4	1.26	1.16	1.68
70	0.32	26	41.0	0.70	1.05	1.25
Mean ± S.E.						
	.47 ± .03	56 ± 6	32.3 ± 3.22	1.39 ± .14	1.16 ± .09	1.68 ± .13

Table 51 (Continued)

Rat no.	Weight	Spleen			Circulating antibodies	
		Total cell count	Nucleated cell	Plaque-forming cell	Agglutinin	Hemolysin
	gm	x 10^7	%	log	log titer	log titer
Experiment IV						
Group Sni						
a	.60	39	41.4			
b	.60	59	55.3			
c	.55	53	62.2			
d	.69	118	46.6			
e	.60	91	44.5			
f	.61	79	41.1			
g	.69	50	35.5			
h	.69	64	64.2			
i	.70	79	59.1			
j	.50	57	68.2			
k	.75	110	78.5			
l	.90	35	39.8			
Mean ± S.E.						
	.66 ± .03	70 ± 8	53.0 ± 3.88			