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Formation of immunoproteins during protein repletion

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FORMATION OF IMMUNOPROTEINS DURING PROTEIN REPLETION

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Usefulness of quantitative measurements of plasma proteins to assess repletion of tissues is suggested by the regularity with which changes in amounts of plasma proteins follow the course of protein depletion (1, 2). Participation of certain proteins of the blood in immune reactions attaches additional importance to an understanding of how factors such as dietary protein may influence formation of plasma proteins.

Net synthesis of protein is controlled by supply of amino acids to cells, simultaneous availability of activated amino acids at sites of protein synthesis within cells, supply of energy for formation of peptide bonds, and rates of synthesis and degradation, or "turnover," of individual proteins.

Whereas cells with an adequate supply of amino acids are usually capable of maintaining a steady state with regard to quantities and proportions of various proteins, the presence of an antigen or antigens, for example, may initiate synthesis of proteins which are distinguishable immunologically from normally occurring proteins. The striking effect of antigenic stimulus on production of antibody is recognized; effects of diet and other factors are less well understood.

In dietary deficiency of protein, synthesis of new protein may not keep pace with catabolism of protein, because some amino acids may be degraded before they can be re-utilized. The result would be a reduction in endogenous supply of amino acids to the pool from which new proteins are synthesized. In an animal depleted of labile proteins, protein in the diet contributes a high proportion of the amino acids in the pool. Therefore,

the protein-depleted animal, which is more sensitive than the normal adult animal to variations in the supply of amino acids from the diet, has been selected as an experimental tool.

Immunological reactions involve fairly homogeneous fractions of protein and are characterized by a high degree of specificity. The possible value of measurement of various immunoproteins as a sensitive index of changes in protein metabolism has been pointed out (3, 4, 5). The practical significance in human nutrition and medicine of an understanding of the influence of diet on mechanisms of resistance is obvious.

The purpose of this study was to evaluate the importance of the quality of dietary protein in synthesis of immunoproteins by protein-depleted adult rats, through measurements of complement, properdin, and anti-sheep hemolysin in serum. These immunoproteins were studied in relation to body weight, nitrogen excretion, gross composition of liver, and concentrations of other proteins of blood at various stages during protein depletion and repletion.

REVIEW OF LITERATURE

Amino acids from dietary proteins may be incorporated directly into individual immunoproteins, but interrelationships of many factors involved in resistance must be considered in interpretation of data concerning synthetic mechanisms in immune processes. With competition between host and agent for nutrients (6, 7), overall resistance of the host can be a function of the nutritional requirements and metabolic patterns of the infectious agents, as well as of the immune reactions of the host. For example, viral infections, in which the agent depends on intracellular nutrients and enzymes of the host, often proceeded less rapidly in undernourished individuals than did bacterial infections (8).

Measurements of "resistance" have reflected, among other things, kind of challenge presented to the host (8, 9), genetic background (10) and nutritional status (11, 12) of the host, the immunological response which was measured (13), and various dietary components (8, 14, 15). Since resisting invasion by foreign organisms involved so many factors, prediction of which mechanisms would predominate in resisting a challenge has not always been possible. Importance of antibody in resistance would depend upon such factors as the relative rates of onset of the disease and release of antibody. "Natural resistance" to an infecting organism, mediated in part by natural proteins which were less specific than antibody, was sometimes independent of ability to form antibody (16).

The following discussion has been limited to the influence of dietary protein on selected immunoproteins of the blood and on susceptibility to experimentally-induced infection.

Antibody and Acquired Immunity

Process of immunization

Introduction of a foreign body, or antigen, into the tissues of an animal initiates the complex process of immunization. Antigenic determinants in proteins or carbohydrates of microorganisms can induce formation of specific antibodies by the host to combine with antigen, thus inactivating the organism and enhancing its removal by phagocytosis.

The primary phase of immunization has been characterized by histological changes in spleen or lymph nodes in preparation for synthesis of antibody (17), followed by appearance of circulating antibody (18) and sensitivity to antigen. In the rat, formation of antibody to intravenously administered antigen was initiated in the spleen (19), while extravascular antigen stimulated lymph nodes to form antibody-producing cells (20). Appearance of plasma cells, which may have originated as reticulum cells or as lymphocytes, usually accompanied release of antibody (21). Subsequent exposure to the same antigen produced an immediate rise in concentration of antibody, in contrast to the lag period which characterized the primary response (22).

The nature of antibody

Most antibodies have behaved electrophoretically as gamma globulins, although some have been associated with other globulin fractions (23, 24, 25). Differences in amino acid content or sequence between normal gamma globulin and antibody may exist, but have not been demonstrated conclusively (26, p. 376). Such characteristics of antibody as firmness of binding to antigen and range of cross reaction with complex antigens may change during

the course of immunization as a result of changes in primary or tertiary structure (27, p. 72).

Schoenheimer et al. (28) concluded that antibodies were newly synthesized molecules, into which labeled amino acids can be incorporated at rates comparable to those of incorporation into other proteins of plasma. If the mechanism for synthesis of the peptide chain of antibody is the same as that for other proteins (29), acquired specificity of globulins may result from changing the tertiary structure near the site of synthesis within the cell (30), from its natural configuration to another relatively stable form. Since, according to Burnet (27, pp. 40-80), evidence concerning persistence of antigen in the tissues has not been conclusive, the possible function of antigen as a template for continued formation of antibody molecules, as suggested by Haurowitz (26, p. 387), is still a matter of conjecture.

The reaction of antibody with antigen has generally required no cofactors, has not been greatly sensitive to temperature, and has been recognized by precipitation or agglutination, or by formation of complexes capable of fixing complement (31, 32).

Effects of dietary protein on antibody formation

Protein As early as 1919, Zilva (33) noted slight decreases in antibody production by rats and guinea pigs fed diets deficient in various vitamins or minerals or containing poor quality or small amounts of protein. Later, in support of his theory that depletion of protein reserves eventually extended to loss of "antibody matrix" and of normal ability to form immune bodies (34), Cannon demonstrated that depletion of nitrogen reserves in growing or adult rabbits by feeding sub-optimal amounts of a low nitrogen diet inhibited formation of antibodies to Eberthella typhosa (35, 36).

Protein-depleted rats also formed less hemolysin than inanition controls (16).

Glabais (37) found that hypoproteinemic adult rats repleted with 18% egg protein for 7 days before injection of sheep cells produced more hemolysin on the 8th day after injection than those similarly repleted with 9% egg protein.

Titers of agglutinating antibody were higher in rats fed diets containing 20% protein (mostly casein) than in those fed either 7 or 40% protein for one month (11). After 4 months, antibody response was the same for rats fed 40 and 20% protein, but 7% was insufficient for the same degree of protection. Sprunt and Flanigan (7) have suggested that interference with synthesis of antibody by a very high intake of protein may result from increased need for vitamins such as pyridoxine, which is important in synthesis of antibody (38, 39).

Small groups of growing rats maintained on diets containing 2 or 18% casein produced approximately equal amounts of agglutinating antibody to Salmonella typhimurium (40), but titers of rats fed 18% protein appeared to fall more rapidly than those of rats fed 2%. Steinbock and Tarver (41) have reported that the rate of turnover of plasma proteins was greater when the protein content of the diet was increased.

Importance in immunity of quality as well as quantity of protein has been suggested by the superiority of 22% casein over 22% protein from beef for formation of antibody to sheep cells by protein-depleted rats (42). In addition, Hodges et al. (43) found that less tetanus or typhoid antibody was synthesized by men who consumed 20 g. of milk protein daily than by those who ate the same amount of protein from egg yolk. The amount of

agglutinin rose with 7-fold increase in intake of milk, but decreased when the quantity of egg was tripled.

Protein status of animals at the time of antigenic stimulation, as well as the kind of dietary protein, has influenced immunological response. A 7-day period of repletion with 22% protein from beef before immunization induced higher titers of complement-fixing antibody than were formed after immunization on the first or third day of repletion; however, titers were decreased slightly in rats fed 22% casein if repletion before injection was extended from 2 to 7 days (42).

Amino acids Ludovici et al. (44) investigated the possibility that methionine could spare pantothenic acid for its role in synthesis of antibody, as well as for other metabolic functions of the vitamin. Addition of 1.4 to 2.7% methionine to a 25% casein diet failed to influence growth or deficiency symptoms of pantothenic acid-deficient rats, but counteracted inhibition of antibody formation noted in pantothenic acid-deficient controls which did not receive supplementary methionine.

When growing rats were fed a diet deficient in both tryptophan and niacin, both growth and agglutinin formation were depressed (45). Addition of niacin restored growth, but only tryptophan enhanced synthesis of antibody. Failure of niacin to improve antibody formation in animals fed a corn diet was confirmed by Wertman and Sarandria (46).

Complement

Components

Antigenic cells sensitized with antibody, in the presence of Ca^{++} and Mg^{++} (47), lyse spontaneously following treatment with complement (48, 49).

"Complement" refers to several protein fractions probably formed in the liver (50) and found in normal and immune serum (51). Four major components have been characterized (31, 52, 53).

The first component, C'1, is a heat labile protein from "midpiece," the insoluble fraction separated from serum at pH 5.5, ionic strength 0.02. Its esterase activity was normally inhibited in serum (54). Binding of activated C'1-esterase to antibody-antigen aggregates, in the presence of Ca^{++} (55), appeared to be the first step in complement fixation (56).

C'2 is a heat labile component present in "endpiece," the soluble fraction at pH 5.5, ionic strength 0.02. C'2 was irreversibly changed by interaction with antigen-antibody complexes with which C'1 and C'4 have combined (49, 57).

C'3 is the last component to participate in lysis of cells, and the only one to react in the absence of divalent cations (55, 58). A euglobulin, C'3 contained at least 3 chromatographically distinct proteins, and was inactivated when formaldehyde, inulin, or zymosan, a carbohydrate from yeast, was added to fresh serum (59).

C'4 was stable at moderate temperatures, but was inactivated by ammonia or hydrazine. Other immunologically active factors in serum were also sensitive to these reagents (60); among these was the beta-1C globulin (61) which was considered to be part of C'3. In addition, Müller-Eberhard (61) has separated a heat labile protein of human serum, called the 11S component, which was capable of precipitating soluble aggregates of gamma globulin in the absence of divalent cations. He has suggested that the 11S component could function as part of complement.

Relative proportions of components of complement varied in different species (62). Recent kinetic studies indicated that various inhibitors (63, 64), temperature, and other conditions of the assay (65, 66) may have determined the rate of disappearance of one or another of the components, and hence limited the extent of the overall reaction in vitro.

Although antibody was thought to be required for lysis of bacterial cells in vitro, complement alone may have had some bactericidal activity. Wardlaw (67) suggested that antibody acted by covering or combining with lipopolysaccharides on the surface of antigenic cells. If these constituents of cell membranes were of such composition that they did not interfere with fixation of components of complement, complement might be able to function in the absence of antibody.

Effect of protein and amino acids on serum complement

Young adult rats fed 20% protein for one month had higher titers of complement than rats fed 40 or 7% protein (11). After 4 months on the diets, the complement titers of groups fed 20 and 40% protein were no longer different, but the titer of the group fed 7% protein remained low.

In both rabbits and guinea pigs, injection of ethionine lowered titers for total complement and for individual components of complement (particularly C'1); simultaneous injection of methionine counteracted the reduction in titer (4, 68).

Complement titers in the human diseases diagnosed as beriberi, ariboflavinosis, and pellagra were lower than those of healthy controls, and increased with therapy (69). However, diminished complement titers in rats deficient in pantothenic acid, thiamin, folic acid, biotin, riboflavin, or pyridoxine (39) were attributed to inanition, because deficiencies of these

vitamins did not influence the rate of regeneration of complement (70) when complement levels had been artificially lowered by intraperitoneal injection of bacterial polysaccharide.

The Properdin System and Natural Immunity

Transient bactericidal activity of fresh serum in the absence of specific antibody has been ascribed traditionally to complement. Recent evidence has pointed to involvement of other factors: bactericidal power of serum was not always related to complement titer (71); resistance to certain organisms was not impaired following injection of antibody against complement (13). Other factors in serum which have recently received attention in this connection include lysozyme (67) and properdin (72).

Characterization of properdin

In attempts to isolate C'3, Pillemer *et al.* (73) partially purified a euglobulin which was present as less than one-half per cent of total protein in normal human serum. This protein, properdin, together with Mg^{++} and factors resembling the 4 components of complement, were collectively referred to as the properdin system. Properdin itself was distinct from complement, phosphatase, lysozyme, and agglutinin to zymosan (74, 75).

Properdin participated in many reactions, including inactivation of viruses (76), lysis of abnormal erythrocytes (77, 78), destruction of bacteria (79).

Requirements for complement and divalent cations, marked temperature dependence, low degree of specificity, and other characteristics distinguished the properdin system from antigen-antibody reactions (73, 79). Properdin was originally described as a naturally-occurring protein which

exhibited behavior different from antibody, yet appeared to serve much the same function, i.e., preparation of antigenic material for fixation of complement.

Several workers (80, 81) have postulated that properdin itself is an antibody to an antigenic determinant common to many cells and particles, including zymosan. Observations that the series of reactions of the 4 components of complement with antibody and with properdin were similar (82) tended to support this view.

The discovery that an antibody-like factor was required for inactivation of properdin by zymosan (83) has helped to clarify the situation. Other reports (84, 85) have implicated antibody in addition to properdin in bactericidal activity of serum. Inhibition of bactericidal action of properdin by certain specific antibodies to bacteria (86) may have represented competition between the recognized antibody and a then-unknown antibody capable of fixing both properdin and complement. Lepow (87) has suggested that properdin be classified as a component of complement.

Lack of correlation between "properdin" values in serum assayed by different methods may be explained in several ways. First, properdin may be a mixture of compounds. Further purification of properdin will decide this point. Second, uncharacterized serum factors may be involved with properdin. The presence of these unmeasurable and no doubt variable participants in the reaction could easily explain differences in results obtained with the same assay procedure in the same laboratory, when different batches of serum reagents were used (88). Third, properdin may exist in bound forms. Sera of guinea pigs and arthritic humans contained a protein which inhibited inactivation of C'3 by properdin-zymosan complex (89, 90). After removal

of the inhibitor by washing the properdin-zymosan complex before addition of complement, guinea pig serum was shown to contain almost as much total properdin as rat serum, in which the inhibitor had not been found.

Significance of properdin measurements

Ginsberg and Wedgwood observed that natural resistance of an animal species to spontaneous infection tended to parallel the titer of available properdin (91). Injection of zymosan or bacterial lipopolysaccharide bound properdin in plasma, stimulated synthesis or release of additional properdin, and eventually caused a temporary rise in titer which was accompanied by increased resistance (92, 93). Susceptibility to infection increased as properdin titer decreased in hemorrhagic shock, while complement titer remained constant (94). The amount of properdin in serum has been altered in various infectious and noninfectious diseases (95, 96).

Effects of diet on properdin titer

DeLuca et al. (97) found that properdin concentrations were increased by additional para-aminobenzoic acid, vitamin E, or vitamin K; thiamin, vitamin D and vitamin B_{12} had less marked influence. Similarly, Wiss et al. (98) noted low properdin titers in rats deficient in pantothenic acid, but not in those lacking thiamin. Properdin titers were depressed by vitamin A deficiency in young rats (99).

Properdin levels were low in growing rats fed only non-essential fatty acids. Inclusion of other fatty acids in the diet stimulated a temporary rise in titer (100).

The effect of dietary protein on properdin titer has not been reported.

Overall Resistance

Although antibody, complement, properdin, and numerous other components of plasma each contribute to resistance, no single factor invariably controls the outcome of host-agent encounters. Susceptibility to infection could be changed without concomitant alteration in ability to form specific antibodies to mouse pneumonia virus (101) or in the pattern of removal of organisms (102). Lowering complement titers by dietary deficiency or by administering anti-complement decreased resistance to some but not all organisms (71, 13). On the other hand, the coincidence of low protein intake, "war edema," and increased occurrence of infectious diseases has been cited as circumstantial evidence of the importance of dietary protein in overall resistance (34). Controlled laboratory experiments, such as those to be reviewed here, are essential to establish the validity of measurements of individual immunoproteins as indices of resistance.

Resistance to bacterial and viral infections

Quantity of protein Dubos and Schaedler (12, 103) noted higher mortality of mice infected with one of 3 strains of bacteria and fed a 5 or 8% casein diet than of those fed 15 or 20% casein. Increasing casein from 8 to 40% of the diet progressively improved survival of mice with viral hepatitis, although optimal growth occurred with 16% casein (104). Resistance against Western equine encephalomyelitis virus was much greater in mice given 18% protein than in those fed none (105). However, substitution of casein for 76% sucrose to produce a "carbohydrate-deficient" diet also lowered resistance to the virus.

Fewer of the rats fed 7% protein than of those given 20 or 40% survived infection with Salmonella parathyphi B (11). Because of spontaneous infections, survival was also low in groups fed 7% protein but not injected with Salmonella. Survival time has been both lengthened (106) and shortened (107) by raising the amount of dietary protein fed to rats with tuberculosis.

Effects of protein on responses to various organisms have differed (9). Mortality of chicks fed mixtures of corn and soybean meal plus methionine was greater with Salmonella gallinarum, but less with Escherichia coli, if their diet contained 30% protein than if it contained 15%. When dietary protein was increased from 15 to 30% at the time of infection, resistance equalled that of chicks given 30% protein throughout. Decreased survival time of chicks infected with Salmonella gallinarum and fed about 30% protein has been confirmed (108, 109).

Kind of protein Data obtained by Schaedler and Dubos (110) permitted ranking of the following proteins (all fed with 0.3% cystine) in approximate decreasing order of protection of growing mice against bacterial infection: 15 or 20% casein, 15% soy alpha protein + rice, 5% casein, 20% soy alpha protein, 15 or 20% wheat gluten ± lysine. Corn alone provided least protection. Earlier, Robertson and Doyle (111) also had reported greater resistance in rats fed casein than in animals given vegetable protein.

Amino acid supplementation Lysine supplementation of a diet containing 20% wheat gluten failed to increase resistance of mice to Klebsiella pneumonia although growth was equivalent to that afforded by casein (110).

Weight loss following injection of small amounts of endotoxin from Escherichia coli, was readily regained by mice fed casein or wheat gluten with lysine and threonine, but not by mice fed gluten without amino acid

supplement (112).

Parasitic infection

Yaeger and Miller (113, 114) studied resistance of rats to Trypanosoma cruzi after supplementation of poor quality protein with those amino acids which were limiting for growth. Addition of lysine or of lysine plus tryptophan to 20% zein decreased parasitemia and mortality to that of rats fed 20% casein, although growth was depressed. Similarly, rats given lysine or methionine with gluten resisted infection better than those given gluten alone or gluten with both amino acids.

A diet low in cystine, Factor 3, and vitamin E lessened survival time of mice but at the same time suppressed Plasmodium berghei infection in mature erythrocytes of the animals (115).

Tumor-bearing Sprague Dawley rats fed 12% casein beginning 2 days after infection with Hemobartonella muris developed hematuria; those fed either 6 or 18% protein showed no symptoms of disease (116).

Increased growth of helminths has been observed in host deficiencies of food energy, protein, vitamin A, thiamin, riboflavin, iron, copper, and cobalt (117), but intakes of protein and vitamin A appeared to be very important (118). Mixtures of proteins which met requirements for amino acids for growth were superior to diets deficient in some amino acids with regard to promotion of resistance to ascarides (119). Specific antigen-antibody interaction, such as occurs in bacterial and viral infections, has not been characterized for helminthic infestation, but such a mechanism may exist.

Interactions of dietary protein with other factors

Several dietary components have influenced the effects of protein on resistance. Hill et al. (14) found that 30% protein in the diet decreased

mortality of Salmonella-infected mice only when fed with an unidentified Salmonellosis resistance factor from wheat. Increasing protein in the absence of the resistance factor decreased survivorship. An excess of vitamins obliterated the effect of increased dietary protein. Nabb and O'Dell (15) first observed that mortality of guinea pigs infected with Salmonella typhimurium was higher when they were fed 30% soy protein than when they were given 30% casein, but later noted the reverse when the composition of the salt mixture was changed to one containing increased amounts of Ca and P. Concentration of protein influenced resistance in mice fed diets containing 5% of methyl esters of the fatty acids of cocoanut oil, but not in animals given 5% lard or 0.2% methyl linoleate (120).

Differences in survival times of Salmonella-infected rats which had been fed different amounts of protein were more marked in immunized than in nonimmunized rats (11).

Both environmental temperature and dietary protein may affect activity of phagocytic cells. At 68° F, 18% casein promoted maximum rate of phagocytosis by blood leukocytes (121).

Sprunt and Flanigan (5) have tentatively suggested a cyclic relationship between susceptibility and "metabolic state" during phases of malnutrition. As animals begin to adapt to an inadequate diet, they become susceptible. Acquisition of ability to utilize labile nitrogen stores induces a temporary resistant state which persists until labile nitrogen is exhausted. Use of less readily mobilized tissue protein for maintenance of vital processes leads to progressive fall in resistance, as tissue wastage continues.

Both quantity and quality of dietary protein influence resistance. Confusion concerning the significance of the relationship may have arisen from lack of recognition of the many factors which interact with dietary protein to influence observed resistance. Dietary components, such as quantity of vitamins, kind of fat, composition of salt mixtures, and presence of Salmonellosis resistance factor, as well as environmental temperature, state of immunity of the host, and capacity of an animal to mobilize tissue protein under the stress of infection all have been shown to alter the effect of dietary protein on resistance.

PROCEDURE

This study consisted of 5 parts. The first 2 were of a preliminary nature, and the results will not be reported in detail. In both Experiments III and IV, animals were placed on experiment at 2 times; the notations IIIa, IIIb, IVa, and IVb have been used to indicate animals studied at approximately the same time. The fifth experiment was an extension of Experiment IV; in Experiment V, animals were depleted for varying periods before injection of antigen.

General Plan

Experiments I and II

Adult male rats were depleted of protein for at least 18 days before injection of 1 ml 0.25% sheep erythrocytes (Figure 1, Table 1). Some of the animals were then fed a diet containing about 8% casein or 8% soy alpha protein with methionine. One group was repleted for 6 days with 8% casein before immunization; other groups were maintained on stock ration or 8% casein or low nitrogen diet throughout the study. Hemolysin and hemagglutinin were measured 4 days (Experiment I) or 6, 9, 12, and 15 days (Experiment II) after injection of antigen. Hepatic nitrogen and fat, and concentrations of hemoglobin and serum nitrogen were also measured.

The results suggested use of the following procedures in subsequent work: 1) inclusion of about 12 animals in each group; 2) use of proteins varying widely in quality; 3) increased dose of antigen; 4) examination of the role of methionine in repletion of proteins of liver and serum; 5) longer periods of protein depletion; 6) frequent measurement of antibody titer around 6 days after immunization.

Figure 1. Summary of experimental plan for stock control, protein depleted, and repleted animals in preliminary Experiments I and II and in Experiments III, IV and V, showing periods of depletion and repletion, time of injection of antigen, and periods of collection of urine for determination of nitrogen excretion

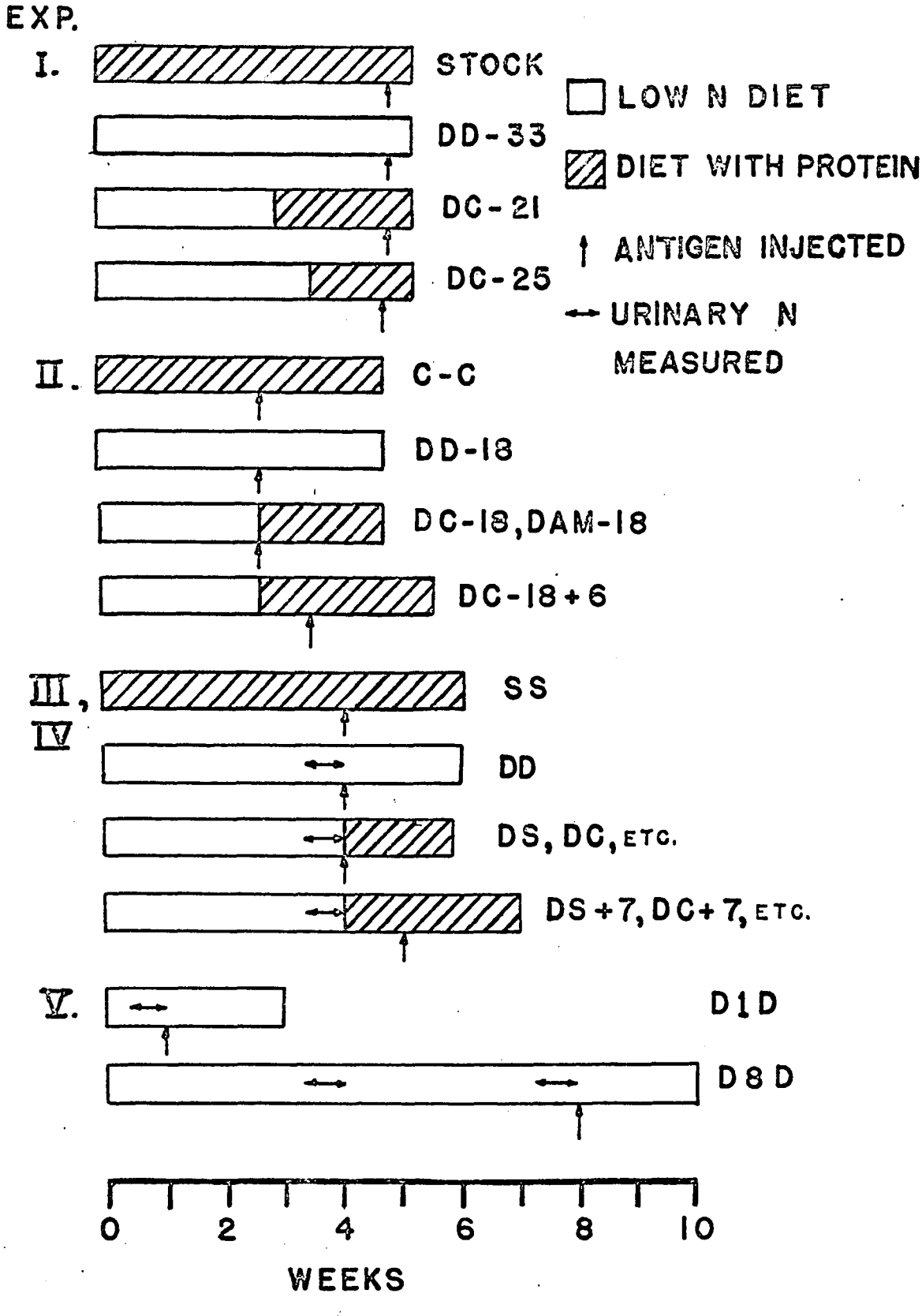
EXP.
I.
STOCK
DD-33
DC-21
DC-25
LOW N DIET
DIET WITH PROTEIN
ANTIGEN INJECTED
URINARY N MEASURED
II.
C-C
DD-18
DC-18, DAM-18
DC-18+6
III, IV
SS
DD
DS, DC, ETC.
DS+7, DC+7, ETC.
V.
D1D
D8D
0
2
4
6
8
10
WEEKS

Table 1. Experimental plan and dietary sources of nitrogen for preliminary Experiments I and II and for Experiments III, IV, and V

Experiment number	Group symbol	Number of rats	Dietary source of N	Days depleted	Days repleted
I	Stock	4	Stock ration	-	-
	DD-33	3	None	33+4[a]	-
	DC-21	3	8% casein	21	12+4[a]
	DC-25	2	8% casein	25	8+4
II	C-C	6	8% casein	-	-
	DD-18	7	None	18+15	-
	DC-18	8	8% casein	18	0+15
	DAM-18	7	8% alpha protein with 0.25% methionine	18	0+15
	DC-18+6	8	8% casein	18	6+15
III	SS	13	Stock ration	-	-
	DD	14	None	28+14	-
	DS; DS+7	12; 13	Stock ration	28	0+14; 7+14
	DC; DC+7	13; 12	9% casein	28	0+14; 7+14
	DCC; DCC+7	12; 12	18% casein	28	0+14; 7+14
	DA; DA+7	13; 13	9% alpha protein	28	0+14; 7+14
	DAM; DAM+7	12; 11	9% alpha protein with 0.25% methionine	28	0+14; 7+14
	DG; DG+7	13; 12	9% wheat gluten	28	0+14; 7+14
	DGL; DGL+7	13; 13	9% wheat gluten with 0.35% lysine	28	0+14; 7+14
IV	DE; DE+7	12; 12	9% egg	28	0+14; 7+14
	DEE+7	11	18% egg	28	7+14
	DAM+7′	6	9% alpha protein with 0.25% methionine	28	7+14
V	D1D	6	None	7+14	-
	D8D	10	None	56+14	-

[a]First figure represents days before injection of antigen; second figure, days after injection.

Experiment III

Adult male rats were fed a low nitrogen diet, and urinary nitrogen excretion was measured during the 24th through 28th days of depletion (Figure 1). After 28 days of nitrogen depletion, animals were repleted with one of the following sources of protein: casein, soy alpha protein with or without methionine, wheat gluten with or without lysine, or a mixture of animal and vegetable proteins (Table 1). Antigen was administered on the 1st or 8th day of repletion, and titers of antibodies to sheep erythrocytes were measured at intervals of 4, 5, 6, 7, 10, and 14 days following injection of antigen. Concentrations of complement and hemoglobin were determined immediately preceding the injection; these components, as well as serum nitrogen, distribution of serum proteins, and properdin, were measured 2 weeks later, at the termination of the experiment. Livers were analyzed for fat and nitrogen. Data from repleted animals were compared with those from depleted rats and from rats fed stock ration throughout the experiment.

Experiment IV

The procedure for Experiment IV was similar to that for Experiment III; 9 and 18% egg protein and 9% soy alpha protein with methionine were the test proteins used for repletion. Animals receiving 9% egg protein were injected on the 1st or 8th day of repletion; those repleted with 18% egg protein were injected on the 8th day (Figure 1, Table 1). Six rats were repleted with alpha protein plus methionine for 7 days and injected with sheep cells on the 8th day, as in Experiment III.

For animals fed egg diets in Experiment IVa, and for those fed Diet AM, hemolysin was titrated 5, 6, 10, and 14 days after injection of sheep eryth-

rocytes; in Experiment IVb, hemolysin was also measured on the 4th and 7th days. Agglutinin was not measured in Experiment IV; urine collections during depletion were made only for animals in Experiment IVa.

Experiment V

Experiment V was a study of the effects of protein depletion for periods longer and shorter than in Experiment III. Rats were treated like Group DD of Experiment III except the depletion period before immunization was 1 or 8 weeks, rather than 4 weeks (Figure 1, Table 1).

Since Experiments IV and V were carried out simultaneously, data for depletion of rats in both experiments may be compared directly.

Care of Animals

Two shipments of 3-month old male albino rats[1] were used in Experiment III. They were kept in this laboratory and fed stock diet until rate of gain stabilized, before protein depletion was begun. Animals in Experiments IV and V were from the colony at Iowa State University, which was established in September, 1962, from breeding stock obtained from the same source and at the same time as the animals used in Experiment III.

Rats were housed in hanging wire cages in an air-conditioned laboratory (24° C) and were weighed 3 times weekly. Food and distilled water were given ad libitum. Diets were stored in a freezer until used, and uneaten food was discarded every other day. Food intake was measured during the last 5 days of depletion (when urine collections were made) and during

[1]Wistar strain, from pathogen-free stock, Simonsen Laboratories, White Bear Lake, Minnesota.

repletion. Food consumption of rats in Experiment IIIa was recorded during the entire depletion period. Rats were fasted for 6 hours before collection of blood for measurement of complement or properdin.

Composition of diets

Semi-synthetic diets Components of semi-synthetic diets are listed in Table 2. Diet D included no intentional source of protein; it contained nitrogen equivalent to about 0.2% protein. Diets for repletion contained either 9 or 18% protein, including any amino acid supplement (calculated as N x 6.25 for alpha protein, gluten, and egg; N x 6.38 for casein).

Semi-synthetic diets were supplemented daily with 500 mg of a mixture containing crystalline vitamins (Table 3), plus 50 mg cod liver oil, and 0.75 mg dl-alpha-tocopherol in 50 mg cottonseed oil.

Stock diet A modified Steenbock diet, which has been used successfully for male rats in a stock colony in this laboratory for several years, was fed to one group (SS) throughout the experiment, and to 2 groups (DS, DS+7) during the repletion period. Diet S included Steenbock XVII mixture (Table 4) plus 150 mg cod liver oil, 15 g fresh ground meat, 20 g raw carrots, and 10 g cabbage weekly.

Collection and storage of blood

Blood was collected at the time of immunization and at intervals thereafter. At autopsy, blood was removed from the abdominal aorta; otherwise a cut was made across the tail, and blood flowed freely into a small centrifuge tube or glass capillary. Animals were anesthetized with ether before injection and with sodium pentobarbital before autopsy, but were given no anesthetic at other times when blood was taken.

Table 2. Composition of semi-synthetic diets used in Experiments III, IV, and V

Component	D	C	CC	A	AM	G	GL	E[a]	EE[a]
	g/100 g diet								
Corn dextrin[a,b]	83.0	72.8	62.7	72.7	72.6	71.6	71.7	69.9 69.7	56.9 56.4
Crisco	10	10	10	10	10	10	10	10	10
Hawk and Oser salts[c]	4	4	4	4	4	4	4	4	4
NaCl	1	1	1	1	1	1	1	1	1
Non-nutritive fiber[c]	2	2	2	2	2	2	2	2	2
Vitamin-free casein[c] (13.90% N[d])	-	10.2	20.3	-	-	-	-	-	-
Soy alpha protein[e] (13.99% N[d])	-	-	-	10.3	10.1	-	-	-	-
Wheat gluten[c] (12.60% N[d])	-	-	-	-	-	11.4	10.9	-	-
Hexane extracted whole egg[c] (10.8-11.0% N[d])	-	-	-	-	-	-	-	13.1 13.3	26.1 26.6
DL-methionine[c]	-	-	-	-	0.25	-	-	-	-
L-lysine monohydrochloride[e]	-	-	-	-	-	-	0.43	-	-

[a]The first figure is for lot 3249C (11.0% N); the second is for lot 3008D (10.8% N). Diets were calculated to give 1.44 or 2.88% N from egg. For other sources of protein, the same lot was used throughout.

[b]Fisher Scientific, Chicago, Illinois.

[c]General Biochemicals, Incorporated, Chagrin Falls, Ohio.

[d]N determined by analysis.

[e]Nutritional Biochemicals Corporation, Cleveland, Ohio.

Table 3. Composition of mixture of crystalline vitamins given daily to animals consuming semi-synthetic diets

Component	Amount mg/rat/day
Thiamin hydrochloride	0.040
Riboflavin	0.060
Pyridoxine hydrochloride	0.040
Calcium pantothenate	0.100
Nicotinic acid	0.500
Folic acid	0.0080
Biotin	0.0010
Vitamin B_{12}	0.00075
Ascorbic acid	1.0
Choline chloride	5.0
Inositol	10.0
Para-aminobenzoic acid	10.0
Dextrin	to make 500.0 mg

Blood was allowed to stand at room temperature until the clot had formed and retracted. If complement or properdin was to be measured, blood was centrifuged twice for 15 minutes at 0 to 5° C; otherwise one 30-minute centrifugation at 5 or 25° C was used. Serum was stored at -30° C.

Collection of urine

During the last 5 days of depletion, rats were housed in cages fitted with metal funnels. Urine was collected in 250-ml flasks containing 50 ml 7% HCl. Each day, after removal of feces and spilled food, funnels were

Table 4. Composition of Steenbock XVII diet mixture

Dietary component	Per cent
Corn meal[a]	48.3
Linseed meal[b]	13.8
Klim[c]	13.8
Wheat germ[d]	8.6
Yeast (unirradiated)[e]	8.2
Yeast (irradiated)[f]	0.4
Casein[c]	4.3
Alfalfa meal[g]	1.7
$CaCO_3$ and trace elements	0.4
NaCl	0.4

[a]Grain storage, Department of Animal Husbandry, Iowa State University.

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

[c]The Borden Company, Browntown, Wisconsin.

[d]General Mills, Incorporated, Chicago, Illinois.

[e]Pabst Sales Company, Chicago, Illinois.

[f]About 135 grams of yeast are spread in thin layers on metal trays and are exposed to ultra violet rays for eleven minutes.

[g]National Alfalfa, Lexington, Nebraska.

rinsed into flasks with distilled water. At the end of the period, cage and funnel were rinsed carefully with warm distilled water; rinsings and urine were made to 1 l and stored at or below 5° C. Recovery of nitrogen from human urine pipetted on cages over a 5-day period was about 98%.

Autopsy

Rats were anesthetized by intraperitoneal injection of about 50 mg of sodium pentobarbital. A midline incision was made to, but not through the diaphragm. Blood was taken from the tail and from the abdominal aorta. The liver was removed, weighed to 0.01 g, and frozen. The lungs were examined, and the amount of abdominal fat was graded +, ++, +++, or ++++.

Serological Techniques

Antibody

Immunization Although earlier experiments (37; Experiments I and II) indicated that a single injection of 1 ml of 0.25% sheep erythrocytes gave a rapid antibody response in rats, 1 ml of a 2% suspension of cells increased the production of antibody. A second injection of sheep cells did not raise titers of hemolysin in rats of the strain used in this study, confirming a report by Heiders et al. (122).

Sheep blood[2] was stored until needed in Alsever's solution, in which cells retain constant susceptibility to lysis for at least 2 months (31, p. 149). Blood cells were washed with 0.85% NaCl at least 3 times, or until the supernatant fluid was clear, and centrifuged in a calibrated tube for exactly 8 minutes at 2000 rpm after the final wash. The volume of

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[2]Probio, Incorporated, Nyack, New York.

packed cells was measured in the tube, and the cells were diluted 1:50 by volume with 0.85% NaCl.

One ml of a fresh 2% suspension of cells was injected with a 24 or 25 gauge hypodermic needle into a lateral caudal vein of a lightly etherized rat[3]. Rubbing the tail lightly with xylene facilitated injection.

Titration of hemolysin All dilutions and washings were made with 0.85% NaCl containing 100 mg $MgCl_2 \cdot 2H_2O$/l. In all titrations, 0.50 ml 2% washed sheep cells were used in a final volume of 2.5 ml. Incubations were carried out in a water bath at 37° C for 30 minutes.

Slightly more guinea pig serum[4] than would supply complement for one day's assays was rehydrated with reconstituting fluid and diluted 1:50. The amount of complement to be used in hemolysin assays was determined each day by adding increasing quantities to tubes containing sheep cells and 2 units of rabbit hemolysin[5]. A 50% unit of complement, the least amount which produced 50% hemolysis, was contained in about 0.10 to 0.15 ml 1:50 guinea pig serum. A unit of rabbit hemolysin for use in titration of complement for assays of hemolysin was the amount which would lyse 50% of the sheep cells in the presence of 0.5 ml 1:50 freshly diluted guinea pig serum after incubation at 37° C.

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[3]Rats 312-329 in Experiment IV were injected in the femoral vein. A small slit made in the skin over the vein was closed by 1 or 2 stitches. The opening healed readily, and there was no indication of infection in these or in other rats on which this procedure was tested.

[4]Dehydrated Bacto-complement, Difco Laboratories, Detroit, Michigan.

[5]Glycerolized rabbit anti-sheep hemolysin, Difco Laboratories, Detroit, Michigan.

Before hemolysin titration, the complement in diluted test serum was inactivated by heating at 56° C for 30 minutes. To 0.5 ml of 2-fold dilutions of inactivated serum were added four 50% units of complement, sheep cells, and 0.85% NaCl. Tubes were shaken, incubated and centrifuged, and the color of the supernatant solution compared visually with that of a freshly-made standard containing 0.25 ml 2% cells lysed completely with saponin. The titer was the reciprocal of the dilution of serum in the tube (or the average of dilutions in 2 tubes) which most nearly matched the color of the standard. A logarithmic transformation was utilized in statistical evaluation of data in order to achieve a more nearly normal distribution of values. Geometric means are reported.

When possible, all samples collected from one animal were analyzed on the same day.

Titration of agglutinin Agglutinin to sheep cells was measured in rats of Experiment IIIa. One-half ml of 0.5% sheep cells was added to each of a series of dilutions of complement-inactivated serum. After 30 minutes at room temperature, solutions were centrifuged for 2 minutes, and the degree of agglutination was estimated after tapping each tube lightly. The least volume of serum which caused definite clumping of cells was the end-point. Titers are reported as reciprocals of arithmetic means of volumes of serum.

Complement

Serum complement was measured on the day the blood was collected. To increasing amounts of 1:20 rat serum in 0.5 ml total volume was added 1 ml of sensitized sheep cells (a 1:1 mixture of a solution of rabbit anti-sheep hemolysin, containing 5 units/ml, and 2% sheep cells). Dilutions were made

with veronal buffer, pH 7.4 (123). Tubes were shaken, incubated at 37° C for 30 minutes, and centrifuged. Endpoints were determined by visual comparison with a 50% hemolysis standard. Titers were recorded as microliters of serum required to lyse half the cells in the incubation mixture, but are reported as reciprocal arithmetic means, or hemolytic units per ml.

Properdin

Properdin was titrated by the procedure of Pillemer et al. (123). Step 1 involved adsorption of properdin in test serum with zymosan in the presence of a constant amount of RP[6] (human serum lacking properdin). Decreasing quantities of 1:16 rat serum (0.25 ml, 0.12 ml, etc.) were added to tubes containing a suspension of 3 mg zymosan plus 0.25 ml RP. The mixtures were diluted to 0.75 ml with veronal buffer, pH 7.4, which was diluted daily from a stock solution and used for all dilutions and washings. Tubes were incubated at 37° C in a water bath for 1 hour, with mixing about every 10 minutes, and then centrifuged for 10 minutes at room temperature.

In Step 2, aliquots of 0.2 ml of the supernatant from Step 1 and 0.06 ml R3 (human serum lacking both properdin and Component 3 of complement)

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[6]RP and R3 reagents, zymosan (lot 7B-340), and purified human properdin were supplied by Mr. Earl Todd, Department of Pathology, Western Reserve University. RP was prepared by incubation of pooled fresh human serum with zymosan at 17° C to remove properdin. R3 was made by similar incubation at 37° C, which resulted in loss of properdin and Component 3 of complement, without significant loss of other components of complement. RP contained about 90 units/ml of complement activity. It lost no more than 15-30 units when incubated with zymosan at 37° C, but was completely inactivated in the presence of zymosan and one unit of purified human properdin. R3 was nonlytic. The quantity of zymosan recommended for use (3 mg) was chosen as the maximum amount which inactivated properdin in the presence of RP without appreciably lowering the titer of Component 3 of complement in the absence of properdin.

were mixed with 1.2 ml 0.7% sensitized sheep erythrocytes, prepared as for complement titrations. After incubation for 30 minutes at 37° C, and centrifugation at low speed for 3 minutes, the per cent lysis in each sample tube was estimated to the nearest 10% by visual comparison of the supernatant fluids with solutions of hemoglobin from saponin-lysed cells representing 20, 40, 60, and 80% lysis. The dilution giving 50% lysis was the endpoint. For example, if 0.2 ml of the supernatants from Step 1 which had contained 0.06 and 0.12 ml of 1:16 rat serum gave 70 and 30% hemolysis, the serum contained 1/2 (0.25/0.06 + 0.25/0.12) X 16 = 48 units of properdin per ml.

Although the quantity of R3 used in Step 2 was not lytic, it represented the amount of serum which had contained two 100% units of complement before treatment; it therefore provided an excess of all components of complement except the 3rd one. Thus titration of the supernatant solution from Step 1 was a measure of the amount of Component 3 remaining after the properdin-zymosan complex had inactivated a quantity of Component 3 proportional to the amount of properdin in the test serum. A unit of properdin was defined as a dilution of serum, one-fourth ml of which, after incubation with 0.25 ml RP containing 90 ± 30 units of Component 3 of complement and 3 mg zymosan, inactivated Component 3 in Step 2 as determined by titration to a 50% endpoint. This unit differs from that proposed by Pillemer et al. (123), which stipulated inactivation of all of Component 3 in the second step, and use of RP containing 120 units of Component 3, a slightly higher titer than that used here. Hence, titers of properdin reported by them for rat serum (25-30 units/ml) were somewhat lower than those reported here (32-64 units/ml).

Several precautions were taken to validate the results. Human serum reagents RP and R3, came from a single pool of serum and were prepared at one time with zymosan from the same lot as that used in all assays. Serum reagents were thawed once, refrozen in small containers, and stored at -100° C[7]. Each day new tubes were thawed for use. With each set of 8 to 20 rat sera which were analyzed together, aliquots from tubes containing only RP or RP plus zymosan were titrated to be sure the serum had satisfactory titer, and that incubation with zymosan had not reduced the titer significantly. Because some variation occurred from day to day, dilutions of 1) a pool of rat serum, 2) serum from a single animal which had been analyzed with the previous group of assays, and sometimes 3) a solution of purified properdin were analyzed with each set of new samples. If the titer of the standard pool of serum was greater than 48 ± 16 units/ml, as occasionally occurred, titers for samples analyzed at the same time were adjusted correspondingly. This led to good agreement between titers for serum from individual animals which were re-analyzed that day, and the values previously obtained for those samples. All samples from Experiment IV were titrated on the same day. Six samples from each of Groups SS and DD of Experiment III which had been analyzed on different days were titrated with sera from animals in Experiment V to facilitate comparisons among these groups. The titers for these 12 animals from Experiment III are shown in Table 5 to illustrate the kind of agreement which was obtained on different days.

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[7]Storage space at -100° C was kindly furnished by the Veterinary Hygiene Department.

Table 5. Replication of serum properdin titers of animals in stock and depletion groups of Experiment III obtained by analysis of the same samples on different days

Group SS			Group DD		
Rat number	First analysis units/ml	Repeat units/ml	Rat number	First analysis units/ml	Repeat units/ml
120	32	32	108	48	48
140	24	32	110	48	64
173	48	48	205	48	48
211	32	32	217	64	64
206	64	64	114	48,64	96
277	64	64	262	64	64

Chemical Procedures

Analyses of blood

Hemoglobin Hemoglobin was measured in blood taken from the tail at time of injection of antigen and at autopsy. Exactly 0.020 ml blood was added to 10.0 ml 0.5% NH_4OH. Optical density at 540 mμ of the resulting oxyhemoglobin solution, times 54.6, equalled grams hemoglobin/100 ml blood. The spectrophotometer had been calibrated with blood of known oxygen capacity.

Serum nitrogen Nitrogen in serum was determined by the Dumas method, using a Coleman automatic nitrogen analyzer. Less than 0.05 ml of serum was transferred into a small aluminum container with a calibrated Lang-Levy constriction pipette, dried at 56° C, and combusted in the presence of CuO at 800° C. Nitrogen gas released upon combustion was flushed through the system with pure CO_2 and measured volumetrically in a calibrated metal syringe after absorption of CO_2 carrier gas in KOH solution. The concentration of nitrogen in the sample was calculated as follows:

$$\text{g N/100 ml serum} = \frac{\text{barometric pressure (mm Hg) x volume } N_2 \text{ (µl) x } 0.0449}{\text{temperature } (^{\circ}K) \text{ x volume of sample (µl)}}$$

Serum proteins Serum proteins were separated by paper electrophoresis in an E-C Apparatus unit containing veronal buffer, pH 8.6, ionic strength 0.10 (124). Samples of 0.010 ml serum were pipetted along a line 7 cm toward the cathode from the center of a 22.5" x 2.5" strip of Whatmann 3mm filter paper which had been wet in buffer and placed in the unit for 30 minutes at 100 volts to equilibrate. Proteins were separated at 300 volts for 8 hours, at a current of 6 ma/strip. Tap water (18° C) was circulated through cooling plates above and below the strips throughout the separation. The paper strips were dried on glass plates at 120° C for 30 minutes, dyed with bromphenol blue for 6 hours, rinsed, and the dye fixed with sodium acetate-acetic acid solution (125). Density of dye was measured with a Photovolt densitometer and recorder, using a 495 mµ filter, at a response setting of "5" to give approximately a logarithmic tracing of the actual density. Areas under the curve were determined by counting marks (made by the pen of an attached integrator) which corresponded to various portions of the curve. Four major fractions were measured. Albumin (probably including some alpha-1 globulin), and alpha and gamma globulins were easily discernible; the portion between alpha and gamma globulins, containing several indistinct peaks, was referred to as "beta" globulin.

Hepatic nitrogen and fat

Livers were ground in a tissue homogenizer and made to a volume of 100 ml in distilled water. From each homogenate, aliquots were analyzed for nitrogen by the Kjeldahl procedure (126). Mercuric oxide, used as catalyst, was reduced by powdered zinc before distillation (127). Ammonia

was collected in boric acid containing methyl red-methylene blue indicator (128) and titrated with standardized 0.1 N HCl.

Fat was extracted from 10-ml aliquots of liver homogenate, using ethyl alcohol, Skellysolve B, and diethyl ether, by a modification of the method of Soderhjelm and Soderhjelm (129). The homogenates were extracted twice in Mojonnier flasks. Extracts were evaporated over a steam bath in tared weighing bottles, which were then heated for 15½ hours at 80° C, cooled and weighed. Fat was calculated by difference.

Urinary nitrogen

Aliquots of 50 ml of diluted urine were analyzed for nitrogen by the Kjeldahl procedure as described previously.

Statistical Analysis

Averages for groups are reported with standard errors of the means in tables, and with standard deviations in figures. Analysis of variance, modified for use with samples of unequal size, was carried out in some cases; specific comparisons between pairs of means were made by the "t" test (130, pp. 358, 268, 90). Values of "t" are reported in the Results; values of F and R for analysis of variance and correlations are included in the Appendix.

RESULTS

Weight, Food Intake, and Nitrogen Excretion in Protein Depletion

During the first week of protein depletion, animals lost an average of 7 to 11% of body weight (Table 6). Animals with greatest initial weight tended to lose more weight during the first week than did smaller animals. Thereafter, weight loss was relatively consistent, averaging about 3% per week. For all studies involving repletion, animals were depleted for 4 weeks; the mean loss of body weight during this 4-week period was 22% for Group DD of Experiment III and 18% for all animals of Experiments IV and V. Group DD, although continued on the low nitrogen diet for a total of 6 weeks, was typical of all groups of animals in Experiment III. The variation in weight loss between experiments may have represented differences in the animals themselves. For Experiment III, the animals were shipped to Iowa State from Simonsen Laboratories. They had been fed a different stock ration and handled differently during their period of growth than had the animals in Experiment IV and V, which were raised in the laboratory in the Food and Nutrition Department.

Food intake, weight, and loss of nitrogen in urine during depletion of 3 typical animals of Experiment III are shown in Figure 2. Numbers 111 and 114 lost body weight and urinary nitrogen rapidly at first, then at a gradually diminishing rate. However, nitrogen excretion by number 116 was erratic, and its total losses of body weight and of urinary nitrogen were greater and its food intake less than that of the other 2 animals.

Table 6. Body weight and urinary nitrogen excretion of some of the rats in Experiments III, IV, and V during protein depletion for 1, 2, 6, 8, and 10 weeks

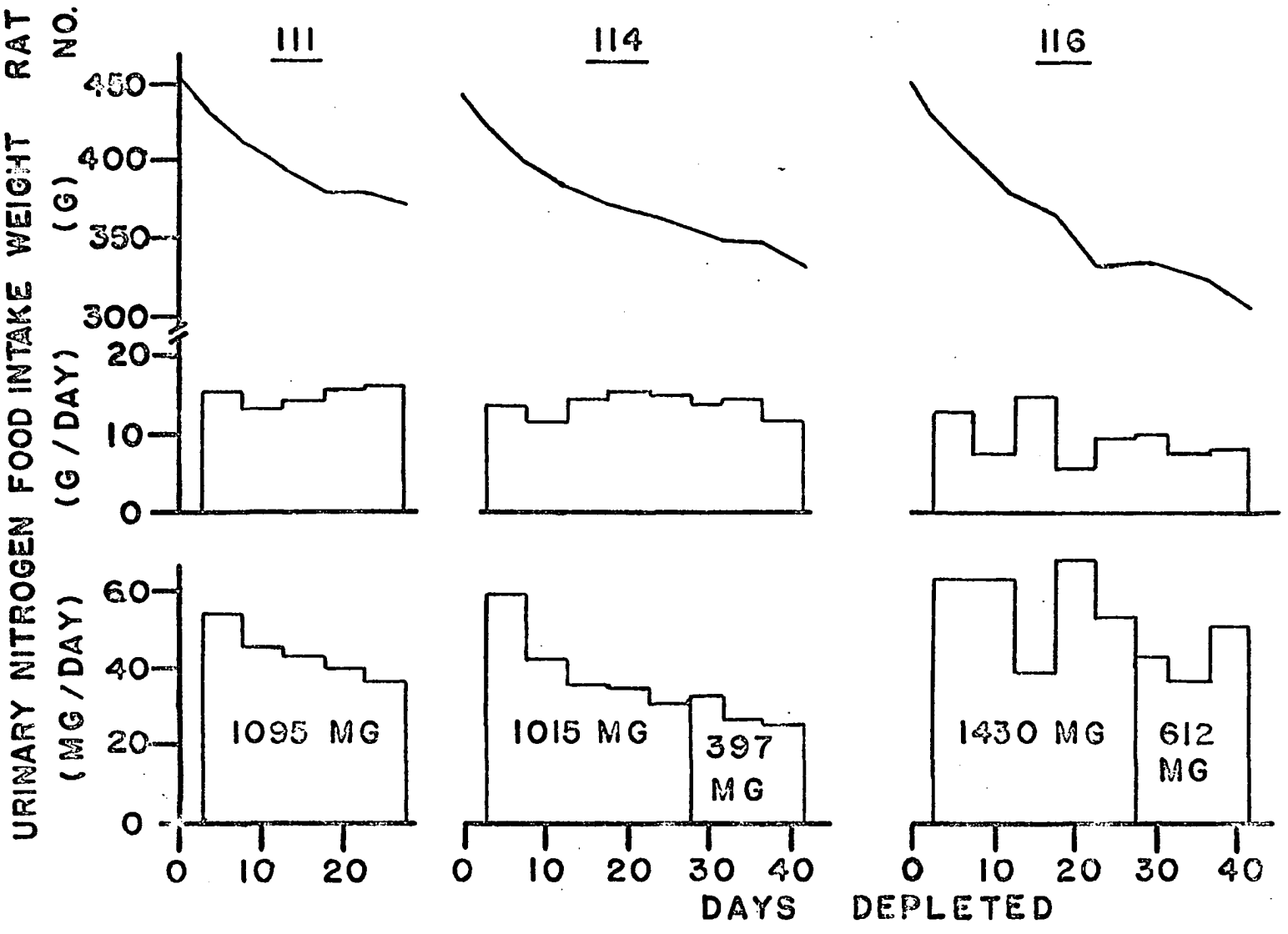
Time depleted	Experiment	Group	Number of rats	Body wt.	Cumulative wt. loss	Urinary N[a]
weeks				g	%	mg/day/ 100 g body wt.
0	III	DD	14	461±9[b]	-	-
	IV	all	41	452±4	-	-
	V	D1D	6	483±16	-	-
		D8D	10	455±14	-	-
1	III	DD	14	410±8	11	-
	IV	all	41	417±4	8	-
	V	D1D	6	432±12	11	17.1
		D8D	10	422±12	7	-
4	III	DD	14	359±8	22	10.4
	IV	all	41	371±3	18	9.1
	V	D8D	10	374±8	18	8.9
6	III	DD	14	332±8	28	-
	V	D8D	10	354±8	22	-
8	V	D8D	10	333±9	27	7.3
10	V	D8D	10	310±8	32	-

[a]Mean for the previous 5 days.

[b]Mean ± standard error.

Data in Figure 2 suggested that variation in quantity of food eaten from time to time during 4 weeks of depletion, as well as the actual amount of food ingested during the period of urine collection, may have influenced nitrogen excretion. In Experiment IIIa the largest amount of nitrogen was excreted by those animals whose voluntary food intake decreased the most between the average for the first 3 weeks and the average for the 4th week of depletion; nitrogen excretion decreased as the mean change in food intake

Figure 2. Weight loss, food intake, and urinary loss of nitrogen of 3 typical animals in Experiment IIIa during protein depletion for 28 or 42 days


RAT NO.
111
114
116
WEIGHT (G)
450
400
350
300
FOOD INTAKE (G/DAY)
20
10
0
URINARY NITROGEN (MG/DAY)
60
40
20
0
1095 MG
1015 MG
397 MG
1430 MG
612 MG
0 10 20
0 10 20 30 40
DAYS DEPLETED
0 10 20 30 40

between these 2 averages became positive (Table 7). Of 119 rats in Experiment III which ate at least 12 g/day during the 5-day period when urine was collected, urinary nitrogen excretion from the 24th through 28th days of depletion varied within the range of 25 to 45 mg/day/rat for 110 animals; animals which ate less than 12 g/day often lost excessive amounts of nitrogen. The amount of urinary nitrogen did not appear to be a function of 1) food intake for either the first 3 weeks or the 4th week, or of 2) weight loss during the 4th week of depletion.

Table 7. Urinary nitrogen excretion in Experiment IIIa during the 24th through 28th days of protein depletion, daily food intake during the first 3 weeks and during the 4th week, and weight loss during the 4th week of depletion of rats which were grouped according to the change in mean weekly food intake between the average for the first 3 weeks and the value for the 4th week of depletion

	Change in food intake from weeks 1-3 to week 4 g/week	Number of rats	Urinary N mg/day	Food intake weeks 1-3 g/day	Food intake week 4 g/day	Weight loss week 4 g/week
A	≤ -21	6	52±5[a]	13.6	9.0	12
B	-20 to -11	9	40±3	14.6	12.1	4
C	-10 to -1	24	37±2	14.6	13.9	8
D	0 to +9	18	34±1	13.4	13.9	14
E	+10 to +19	12	32±1	13.7	15.7	10
F	≥ +20	2	30±4	10.0	13.7	11

[a]Mean ± standard error.

Gross Observations During Protein Repletion

Weight and food intake

Weight gain was influenced by kind and amount of protein and by amino acid supplementation (Table 8). Relative values of diets in promoting weight gain were similar after 1, 2, or 3 weeks of repletion. For illustration, data for weight gain during 3 weeks of repletion are shown in Figure 3. Casein (C) and egg protein (E), as 9% of the diet, were the most efficiently utilized sources of nitrogen for restoring body weight. Doubling the percentage of casein (CC) or of egg (EE) in the diet increased weight gain, but reduced nitrogen efficiency. Addition of methionine to soy protein or of lysine to wheat gluten improved food intake, weight gain, and nitrogen efficiency.

None of the groups in Experiment III gained significantly during the 3rd week of repletion; in contrast, rats in Experiment IV, which had lost less weight during depletion and which ate more during repletion than did animals in Experiment III, continued to gain through the 3rd week of repletion. Nevertheless, their rate of gain decreased in the 3rd week of repletion. As a result nitrogen efficiency calculated for 3 weeks was generally less than for 2 weeks, except with Diet A, which did not support weight gain at any time.

Autopsy

Abdominal fat The quantity of abdominal fat was reduced by protein depletion for 6 or 10 weeks (Table 8), but was restored by feeding S or E for 2 weeks. When rats were fed alpha protein or wheat gluten supplemented with the limiting amino acid, both food intake and fat deposition increased. After 3 weeks, only those rats fed A or G had less fat than stock controls.

Table 8. Weight change, food intake, and estimate of efficiency of utilization of nitrogen for body weight gain during repletion of rats in Experiments III and IV, and size of abdominal fat deposits at autopsy of rats in Experiments III, IV, and V

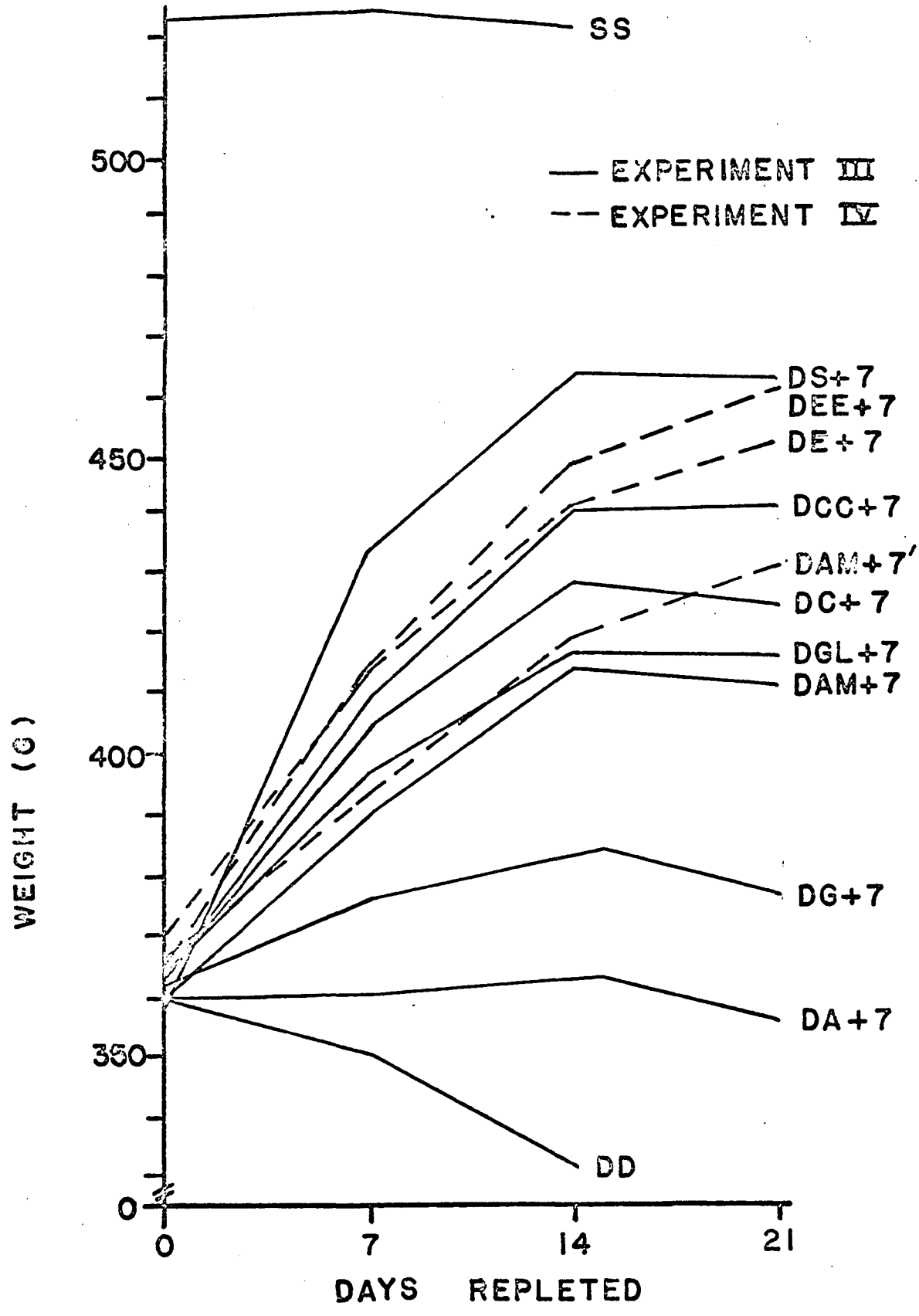
Experiment	Group	Weight change		Food intake	Total N intake	Estimate of N efficiency[a]	Abdominal fat[b]
		g/2 wks.	g/3 wks.	g/wk.	g		
III	SS	-3±6[c]		-	-	-	3.1±0.2
	DD	-27±2		86±3	0.04	0	2.2±0.2
	DS	+91±8		-	-	-	3.2±0.2
	DS+7		+106±7	-	-	-	2.8±0.2
	DC	+47±6		123±5	3.5	21	2.2±0.2
	DC+7		+63±5	133±2	5.6	18	3.0±0.2
	DCC	+60±5		120±4	6.8	13	2.6±0.2
	DCC+7		+79±4	126±3	10.7	11	3.1±0.2
	DA	-13±4		91±6	2.6	5	2.5±0.1
	DA+7		-3±3	97±4	4.1	9	2.2±0.2
	DAM	+36±5		123±7	3.5	18	2.6±0.2
	DAM+7		+57±6	125±4	5.3	18	3.0±0.2
	DG	+11±4		114±6	3.3	12	2.7±0.2
	DG+7		+15±3	118±3	5.1	11	2.5±0.2
	DGL	+44±5		130±6	3.7	19	2.9±0.1
	DGL+7		+52±8	129±5	5.6	16	2.9±0.2
IV	DE	+68±7		132±6	3.8	25	3.2±0.1
	DE+7		+83±6	128±5	5.5	22	3.2±0.2
	DEE+7		+98±7	125±4	10.8	13	3.0±0.2
	DAM+7'		+65±7	136±6	5.9	18	3.2±0.3
V	D1D	-	-	-	-	-	3.0±0.4
	D8D	-	-	-	-	-	1.6±0.2

[a](Total body weight restored and weight lost by Group DD) ÷ N consumed (g); continued loss of weight at the rate of 14 g/week for Group DD was assumed in calculations for "+7" groups.

[b]Graded +, ++, +++, ++++.

[c]Mean ± standard error.

Figure 3. Weight changes of rats in Experiments III and IV during 3 weeks of protein repletion and of animals continued on stock diet or depletion diet for 2 weeks


SS
EXPERIMENT III
EXPERIMENT IV
DS+7
DEE+7
DE+7
DCC+7
DAM+7'
DC+7
DGL+7
DAM+7
DG+7
DA+7
DD
500
450
400
350
0
WEIGHT (G)
0
7
14
21
DAYS REPLETED

Lungs Respiratory difficulties were often noted for brief periods, but were no more severe in animals in which obvious lung damage was observed at autopsy than in otherwise healthy animals. Large infected areas, which were seen in lungs of 2% of the animals in Experiment III, 14% of those in Experiment IV, and 25% of those in Experiment V, did not appear to be related to diet.

Liver

After 3 weeks of depletion, weight of fat-free liver was 40% less, and nitrogen content over 50% less than values for stock controls (Figure 4, Table 9). Thereafter only slight decreases in fat-free weight and in nitrogen were noted although the depletion regimen was continued.

Stock ration (S) was superior to all other diets in promoting formation of fat-free tissue of liver during the first 2 weeks of repletion (Table 9). However, after 2 weeks, weight of fat-free liver of rats fed CC, AM, GL, or E was significantly greater than that of depleted animals; a similar effect was achieved after 3 weeks of repletion with C, but was not observed in rats fed G or A even after 3 weeks (Table 10).

The livers of rats fed S, EE, and CC contained the most nitrogen; those of rats fed GL, C, E, and AM contained intermediate amounts, with E perhaps more efficient in restoring nitrogen than the other diets if the difference between responses to E and AM in Experiment IV is real. Livers of rats given A and G contained the least amounts of nitrogen among the repleted groups, but more than that of animals continued on depletion diet.

Figure 4. Weight, amount of fat, amount of fat-free tissue, and total nitrogen content of liver after protein depletion for 0, 3, 6, and 10 weeks

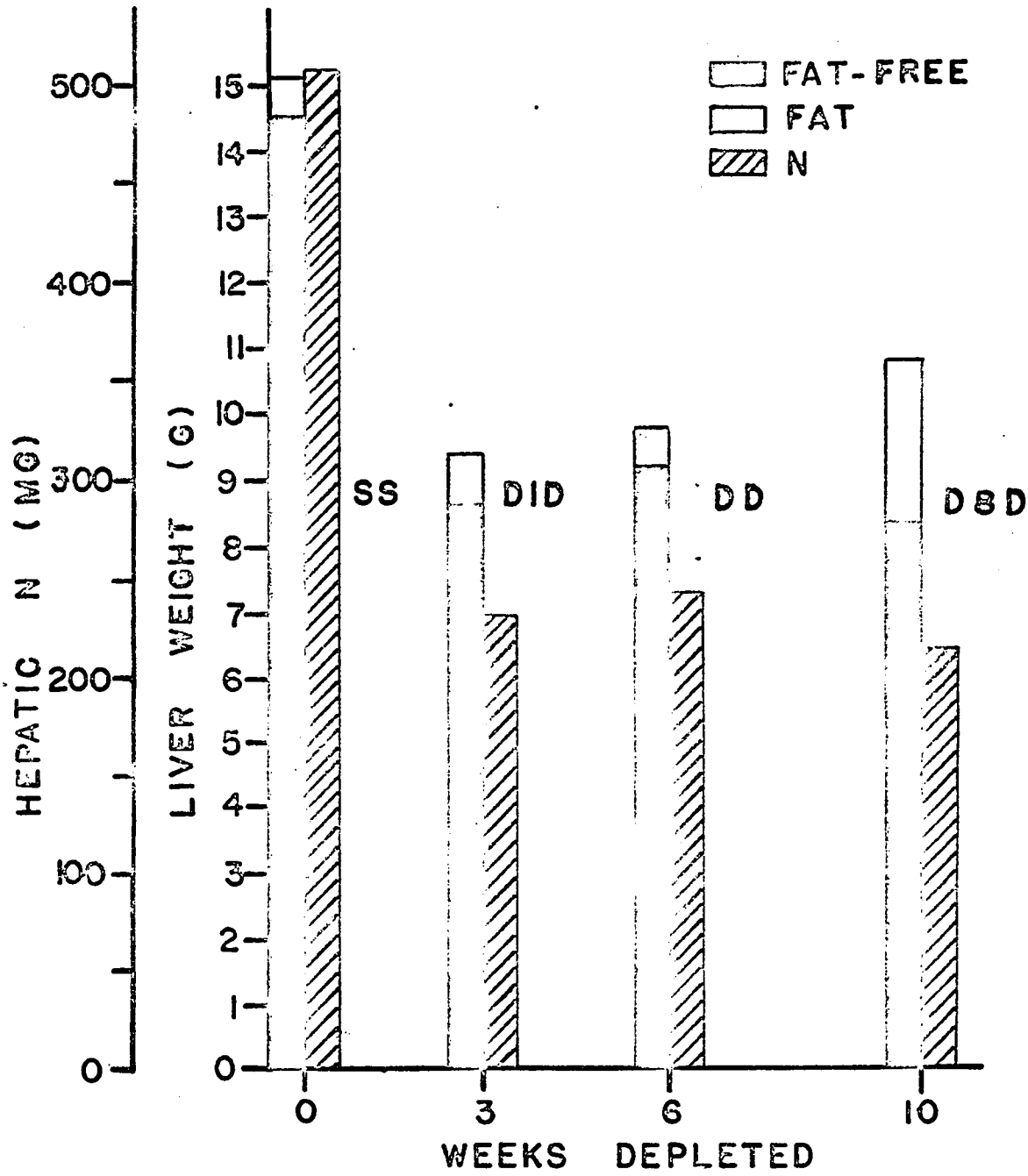

FAT-FREE
FAT
N
HEPATIC N (MG)
LIVER WEIGHT (G)
500
400
300
200
100
0
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
SS
DID
DD
DBD
0
3
6
10
WEEKS DEPLETED

Table 9. Nitrogen and fat content of the liver and relationship of hepatic nitrogen to body weight in animals repleted for 2 or 3 weeks with various proteins, in stock controls and in animals depleted for 3, 6, or 10 weeks

Experiment	Group	Fat-free liver	Total hepatic N	Mg hepatic N	Fat in whole liver
		g	mg	100 g body wt.	%
III	SS	14.5±.8[a]	508±22	97±2	4.3±.2
	DD	9.2±.6	240±13	73±4	4.5±.4
	DS	13.2±.7	479±20	106±3	4.2±.1
	DS+7	12.4±.6	450±18	97±2	4.4±.1
	DC	10.4±.4	354±16	87±4	4.6±.1
	DC+7	11.0±.5	379±20	89±4	4.9±.2
	DCC	11.2±.4	404±13	97±4	4.5±.2
	DCC+7	11.3±.5	402±16	91±3	4.6±.2
	DA	9.4±.4	298±10	84±2	3.9±.2
	DA+7	9.4±.5	300±13	84±3	4.6±.5
	DAM	11.4±.6	358±15	90±3	5.3±.5
	DAM+7	11.0±.4	370±12	90±3	5.0±.3
	DG	10.3±.6	334±18	88±3	4.8±.3
	DG+7	9.2±.4	301±14	79±3	5.2±.4
	DGL	10.8±.4	347±7	86±2	5.5±.3
	DGL+7	10.9±.4	381±11	92±3	6.2±.6
IV	DE	10.8±.4	350±14	78±2	4.9±.2
	DE+7	11.4±.5	372±13	82±2	4.7±.2
	DEE+7	11.5±.3	399±8	86±1	4.6±.2
	DAM+7	10.4±.3	331±8	77±2	6.0±.4
V	D1D	8.6±.4	233±12	60±2	7.9±1.4
	D8D	8.4±.5	200±11	63±3	21.0±2.7

[a]Mean ± standard error.

Table 10. Values of "t" for fat-free weight and per cent of hepatic fat and for ratio of hepatic nitrogen to body weight

Groups compared		Fat-free weight	Mg N / 100 g B.W.	% Fat
SS	DD	5.4**[a]	5.6**	0.5
DD	D1D	0.6	2.5*	3.2**
DD	D8D	1.1	2.2*	7.0**
D1D	D8D	0.4	0.8	3.6**
DC	DC+7	1.0	0.4	1.1
DC	DCC	1.4	2.0	0.4
DC+7	DCC+7	0.3	0.4	0.9
DCC	DCC+7	0.1	1.4	0.3
DA	DA+7	0.0	0.0	1.4
DA	DAM	3.0**	2.0	2.7*
DA+7	DAM+7	2.3*	1.4	0.7
DAM	DAM+7	0.6	0.0	0.5
DG	DG+7	1.5	1.9	0.8
DG	DGL	0.7	0.6	1.6
DG+7	DGL+7	2.7*	2.9**	1.4
DGL+7	DGL+7	0.2	1.7	1.1
DE	DE+7	0.9	1.4	0.7
DE+7	DEE+7	0.1	1.9	0.4
DAM+7'	DE+7	1.3	1.9	3.4**
DAM+7'	DEE+7	2.5**	4.3**	3.7**
DAM+7	DAM+7'	0.9	3.3**	2.0
SS	DS	1.2	2.3*	0.4
SS	DS+7	2.0	0.0	0.4
SS	DC	4.6**	2.3*	1.4
SS	DC+7	3.5**	1.8	2.0
SS	DCC	3.6**	0.0	0.7
SS	DCC+7	3.3**	1.6	1.1
SS	DA	5.8**	4.4**	1.6
SS	DA+7	5.3**	3.2**	0.6
SS	DAM	3.0**	2.0	1.9
SS	DAM+7	3.5**	1.9	2.1
SS	DG	4.3**	2.2*	1.3
SS	DG+7	5.6**	4.4**	2.0
SS	DGL	4.1**	3.7**	3.5**
SS	DGL+7	3.9**	1.3	3.1**

[a]Probabilities of less than 0.05 indicated by (*); probabilities of less than 0.01 by (**).

Table 10. (Continued)

Groups compared		Fat-free weight	Mg N / 100 g B.W.	% Fat
DD	DS	4.4**	7.0**	0.7
DD	DS+7	3.8**	6.1**	0.2
DD	DC	1.6	2.8*	0.3
DD	DC+7	2.4*	3.0**	0.9
DD	DCC	2.8*	4.8**	0.0
DD	DCC+7	2.6*	4.1**	0.2
DD	DA	0.3	2.9**	1.5
DD	DA+7	0.3	2.3*	0.2
DD	DAM	2.7*	3.9**	1.3
DD	DAM+7	2.3*	3.7**	1.0
DD	DG	1.4	3.1**	0.6
DD	DG+7	0.1	1.2	1.2
DD	DGL	2.2*	3.4**	2.1*
DD	DGL+7	2.4*	4.1**	2.4*

The ratio of hepatic nitrogen to body weight had decreased significantly by the end of the 3rd week of protein depletion, but did not change further from the 3rd to the 10th week. The initial ratio was restored after 2 weeks of repletion with CC or AM, and was greater than normal in animals repleted with S. After 3 weeks of repletion, ratios for rats fed GL, C, and E were normal, but those of rats fed A and G remained low. Groups DAM+7 and DAM+7' produced ratios of hepatic nitrogen to body weight which were 1.23 and 1.24 times as high as those of Group DD of Experiment III and of depleted groups in Experiment V. Ratios for egg-fed animals and for rats repleted with S or CC were all about 1.3 times as high as ratios for comparable depleted animals.

Fat content of the liver was higher after 3 weeks of depletion than in stock controls, but equaled the initial amount after 6 weeks, and finally rose to very high concentrations after 10 weeks of depletion (Table 9).

However, the difference in fat content of the groups repleted with AH in Experiments III and IV suggested that the apparent difference between Groups SS and DD of Experiment III and Group D1D of Experiment V may not have been due to protein depletion. In Experiment III, only those rats repleted with GL had significantly more hepatic fat than animals maintained on stock ration.

Major Proteins of Blood

Hemoglobin

The concentration of hemoglobin was not significantly lowered until after 4 to 6 weeks of protein depletion (Tables 11, 12). Hemoglobin concentration fell during the first 2 weeks of repletion and rose slightly during the 3rd week. These alterations in hemoglobin concentration in depletion and in repletion may have resulted in part from changes in blood volume. In rats depleted to about the same degree as those in Experiments III and IV, Weimer (1) noted a decrease in hematocrit corresponding to an increase of 10 to 15% in blood volume; hematocrit did not return to normal until animals were repleted to more than 90% of initial weight. After 3 weeks of repletion, rats in Experiments III and IV weighed between about 70 and 90% of their original weights.

Analysis of variance revealed no significant differences among groups repleted with semi-synthetic diets in Experiment III, although the change in mean concentration in rats fed G appeared to be greater than in animals fed other diets. After 2 weeks of repletion, concentrations for all groups except DG were greater than for DD (Table 12). Concentration of hemoglobin

Table 11. Concentrations of hemoglobin in rats after repletion for 0, 7, 14, and 21 days with various proteins, and changes in hemoglobin concentration occurring during the first 2 weeks of repletion

Experiment	Group	Concentration of hemoglobin after repletion[a]				Change in concentration
		0 days	7 days	14 days	21 days	0 to 14 days
III	SS	14.2±.20		12.7±.38		-1.5
	DD	12.9±.42		10.1±.34		-2.8
	DS	13.6±.22		11.9±.52		-1.7
	DS+7		12.2±.26		11.9±.41	
	DC	13.4±.19		11.4±.30		-2.0
	DC+7		12.4±.28		12.0±.38	
	DCC	13.2±.35		11.4±.42		-1.8
	DCC+7		12.6±.19		11.5±.68	
	DA	13.1±.22		10.4±.52		-2.7
	DA+7		12.7±.22		11.6±.43	
	DAM	13.5±.23		11.2±.27		-2.3
	DAM+7		12.5±.22		11.3±.41	
	DG	13.8±.25		10.0±.55		-3.8
	DG+7		12.4±.19		11.2±.25	
	DGL	13.7±.34		11.4±.19		-2.3
	DGL+7		12.3±.21		11.6±.46	
IV[b]	DE	13.8±.31		11.7±.15		-2.1
	DE+7		12.6±.15		12.9±.23	
	DEE+7		12.5±.15		13.1±.15	
	DAM+7		12.6±.22		12.8±.18	
V	D1D	14.8±.24		12.6±.32		
	D8D	12.0±.35		10.9±.37		

[a]Or after immunization, for groups which were not repleted.

[b]Hemoglobin not measured at injection in Experiment IVa.

Table 12. Values of "t" for comparisons of values for serum nitrogen, hemoglobin, and concentrations of 4 fractions of serum proteins

Groups compared		Serum N	Hemoglobin at autopsy	Albumin	α-globulin	β-globulin	γ-globulin
SS	DD	7.4**[a]	5.1**	2.4*	2.9*	1.3	1.6
DD	D1D	0.4	4.4**	2.1	0.0	0.2	3.1**
DD	D8D	2.6*	1.6	3.2**	0.2	2.2*	0.2
D1D	D8D	3.0**	3.1**	0.9	0.4	2.2*	3.5**
DC	DC+7	1.4	1.3	0.6	1.5	0.0	0.3
DC	DCC	1.1	0.0	1.1	2.6*	0.2	1.6
DC+7	DCC+7	0.0	0.6	0.0	0.8	0.3	0.2
DCC	DCC+7	0.0	0.1	0.6	1.5	0.5	1.1
DA	DA+7	1.3	1.8	0.2	2.1*	0.4	0.1
DA	DAH	2.5*	1.3	0.2	1.7	0.3	0.4
DA+7	DAH+7	1.7	0.5	0.2	0.8	0.9	0.1
DAH	DAH+7	0.4	0.2	0.1	0.5	0.4	0.5
DG	DG+7	1.5	1.9	1.7	1.0	0.1	1.7
DG	DGL	4.1**	2.4*	1.2	0.1	0.1	0.6
DG+7	DGL+7	1.5	0.7	0.7	1.4	0.2	0.7
DGL	DGL+7	0.8	0.4	0.0	0.6	0.1	1.5
DE	DE+7	1.4	4.4**	2.7*	2.1*	3.0**	0.8
DE+7	DEE+7	0.4	0.7	0.5	0.4	0.5	1.1
DAH+7'	DE+7	3.6**	0.3	2.5*	0.9	0.2	0.3
DAH+7'	DEE+7	3.2**	0.1	2.2*	0.9	0.2	1.1
DAH+7' (IV)	DAH+7 (III)	2.2*	2.7*	2.0	1.1	1.4	0.2

[a]Probabilities less than 0.05 are indicated by (*); probabilities less than 0.01, by (**).

Table 12. (Continued)

Groups compared		Serum N	Hemoglobin at autopsy	Albumin	α-globulin	β-globulin	γ-globulin
SS	DS	0.0	1.3	0.6	1.4	0.9	0.4
SS	DS+7	0.5	1.4	1.7	0.6	1.7	0.2
SS	DC	1.1	2.7*	1.1	1.5	1.9	0.0
SS	DC+7	0.4	1.3	0.7	0.4	1.7	0.3
SS	DCC	0.3	2.3*	0.1	0.9	1.2	1.4
SS	DCC+7	0.4	1.6	0.6	0.6	1.6	0.0
SS	DA	4.5**	3.5**	1.2	2.8*	0.8	1.2
SS	DA+7	3.8**	1.9	1.3	0.6	0.5	1.2
SS	DAM	2.3*	3.2**	1.1	0.4	1.1	0.9
SS	DAM+7	1.6	2.5*	1.1	0.1	1.3	1.2
SS	DG	5.2**	4.0**	1.4	2.1*	0.5	0.6
SS	DG+7	3.7**	3.2**	0.4	3.5**	0.4	2.1*
SS	DGL	1.4	3.0**	0.3	2.1*	0.6	0.0
SS	DGL+7	1.9	1.8	0.3	0.8	0.5	1.6
DD	DS	8.1**	3.0**	2.2*	1.8	0.1	3.1**
DD	DS+7	7.5**	3.4**	1.3	1.9	0.5	1.9
DD	DC	5.0**	2.9*	1.7	1.6	0.5	1.9
DD	DC+7	7.3**	3.8**	2.1*	2.7*	0.5	1.8
DD	DCC	5.4**	2.4*	2.2*	3.8**	0.2	0.3
DD	DCC+7	7.3**	1.9	1.6	2.1	0.7	1.3

Table 12. (Continued)

Groups compared		Serum N	Hemoglobin at autopsy	Albumin	α-globulin	β-globulin	γ-globulin
DD	DA	2.1*	0.5	1.3	0.4	0.5	0.7
DD	DA+7	3.9**	2.8*	1.0	2.3*	0.9	0.6
DD	DAM	5.2**	2.5*	1.5	1.9	0.2	1.0
DD	DAM+7	4.9**	2.3*	1.3	2.9**	0.2	0.5
DD	DG	2.4*	0.2	0.6	0.8	0.7	1.1
DD	DG+7	3.8**	2.5*	2.8*	0.2	0.8	0.0
DD	DGL	6.5**	3.2**	2.4*	0.9	0.8	1.5
DD	DGL+7	4.3**	2.6*	2.2*	1.3	0.5	0.1

after 4 weeks of depletion in Experiment IV was higher than in Experiment III, and was more nearly maintained between the 4th and 6th weeks of the experiment.

Serum nitrogen

Concentration of serum nitrogen decreased more in the first 3 weeks of depletion than between the 3rd and 6th or 3rd and 10th weeks (Tables 12, 13). When animals which had been depleted 4 weeks were repleted for 2 weeks with C, CC, GL, or S, serum nitrogen concentrations equaled those of stock controls which had not undergone depletion; after 3 weeks of repletion, animals fed AM also had attained this concentration of nitrogen. Supplements of lysine to gluten and of methionine to alpha protein resulted in significantly different values for serum nitrogen after 2 weeks but not after 3 weeks, when compared with the values obtained with unsupplemented proteins. However, values for groups fed A or G for 3 weeks were still significantly lower than for stock controls. Egg protein effected changes similar to those seen with equal quantities of dietary casein, and, like casein, was superior to alpha protein plus methionine.

Concentrations of serum nitrogen increased at about the same rate as did total hepatic nitrogen (Figure 5). In Experiment III concentrations of serum nitrogen and of hemoglobin at autopsy were related after repletion for 2 weeks but not after 3 weeks.

Part of the increase in serum nitrogen in repletion may not have been protein. Henderson et al. (131) have reported that concentrations of most amino acids were decreased during protein depletion, but Steele et al. (132) observed no relationship between quantity of dietary protein and concentra-

Table 13. Concentrations of total nitrogen and of 4 fractions of proteins in serum of rats repleted for 2 or 3 weeks with various proteins, of stock controls and of animals depleted for 3, 6, and 10 weeks

Experiment	Group	Number of rats	Serum N g/100 ml	Albumin g/100 ml[a]	Alpha globulin g/100 ml	Beta globulin g/100 ml	Gamma globulin g/100 ml
III	SS	12	.94±.02[b]	2.42±.20	1.37±.07	.76±.11	1.34±.16
	DD	13	.78±.02	1.84±.14	1.04±.09	.95±.10	1.06±.07
	DS	11	.94±.01	2.28±.14	1.24±.07	.94±.17	1.42±.10
	DS+7	12	.93±.01	2.05±.07	1.30±.10	1.02±.11	1.39±.16
	DC	12	.91±.02	2.16±.11	1.22±.07	1.02±.09	1.34±.13
	DC+7	12	.95±.02	2.26±.14	1.42±.11	1.02±.11	1.29±.11
	DCC	11	.95±.03	2.45±.24	1.45±.05	.98±.14	1.09±.06
	DCC+7	11	.95±.02	2.25±.22	1.30±.09	1.08±.16	1.35±.22
	DA	13	.83±.02	2.11±.15	1.09±.07	.88±.10	1.13±.07
	DA+7	12	.86±.01	2.07±.17	1.31±.08	.83±.10	1.12±.08
	DAM	12	.89±.02	2.15±.15	1.32±.12	.92±.10	1.17±.09
	DAM+7	10	.90±.02	2.13±.18	1.39±.07	.98±.13	1.11±.09
	DG	13	.83±.01	2.01±.22	1.14±.09	.84±.12	1.22±.13
	DG+7	11	.86±.01	2.55±.22	1.02±.07	.83±.12	.96±.07
	DGL	13	.91±.01	2.34±.14	1.15±.08	.85±.09	1.34±.18
	DGL+7	13	.89±.02	2.35±.18	1.25±.14	.86±.15	1.05±.09

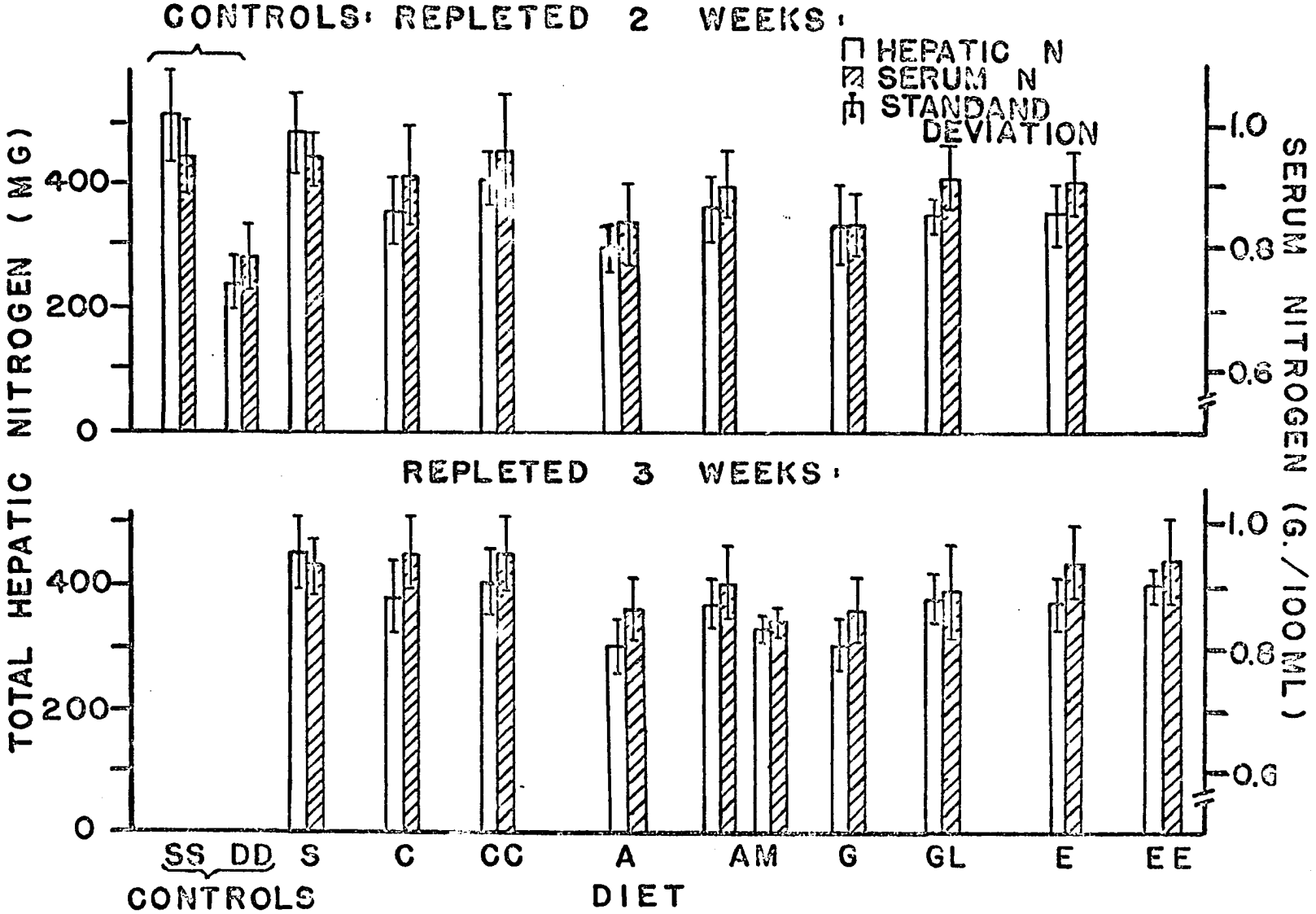
[a]Concentration of serum nitrogen x % of serum protein x 6.25; since no correction for non-protein nitrogen was made, the figures are slightly higher than the actual concentrations.

[b]Mean ± standard error.

Table 13. (Continued)

Experiment	Group	Number of rats	Serum N g/100 ml	Albumin g/100 ml[a]	Alpha globulin g/100 ml	Beta globulin g/100 ml	Gamma globulin g/100 ml
IV	DE	12	.90±.01	2.27±.07	1.16±.06	1.00±.07	1.20±.08
	DE+7	12	.93±.02	2.00±.07	1.39±.09	1.31±.08	1.12±.06
	DEE+7	11	.94±.02	2.06±.10	1.35±.06	1.25±.10	1.22±.07
	DAM+7'	5	.84±.01	1.69±.07	1.26±.09	1.28±.16	1.09±.06
V	D1D	6	.79±.02	1.37±.09	1.04±.05	.98±.07	1.55±.18
	D8D	10	.73±.01	1.25±.09	1.02±.07	1.22±.07	1.04±.04

Figure 5. Mean total hepatic nitrogen and concentration of serum nitrogen of animals repleted for 2 or for 3 weeks with various proteins, of stock controls, and of animals which were depleted for 6 weeks


CONTROLS: REPLETED 2 WEEKS:
HEPATIC N
SERUM N
STANDAND DEVIATION
TOTAL HEPATIC NITROGEN (MG)
SERUM NITROGEN (G./100ML)
400
200
0
1.0
0.8
0.6
REPLETED 3 WEEKS:
SS DD S C CC A AM G GL E EE
CONTROLS
DIET

tion of amino acids in blood. Changes in concentration of non-protein nitrogen, which normally constitutes only about 5% of the total nitrogen of serum, probably did not account for all the differences in serum nitrogen, because these differences were as great as 10 to 15% in some cases.

Serum proteins

Concentrations of serum proteins, in g/100 ml serum, were estimated from the concentrations of total serum nitrogen and the per cent of total protein in a particular fraction. Concentrations of serum albumin and alpha globulin decreased after 3 weeks of depletion, and that of gamma globulin after 6 weeks, while concentration of beta globulin progressively increased (Tables 12, 13). Because variability was large, most differences among means for groups of repleted animals were not statistically significant. Diets containing gluten appeared to stimulate formation of more albumin than did those containing casein or alpha protein, while casein generally favored formation of globulins. When values for casein-fed rats relative to those of Group DAM+7 in Experiment III were compared with values for egg-fed animals relative to those of DAM+7' in Experiment IV, egg protein appeared to be equivalent to casein for formation of all fractions except possibly gamma globulin.

Immunoproteins

Complement

Total serum complement was titrated at the time of injection of antigen and 2 weeks afterward in Experiments IIIb, IV, and V. In protein depletion, titers of complement did not appear to change appreciably until after 10 weeks (Tables 14, 15). The mean titers of all animals depleted for 4 weeks

Table 14. Titers of complement at the times of injection of antigen and titers of complement and properdin at autopsy

Experiment	Group	Complement titer at injection units/ml		Complement titer at autopsy units/ml		Properdin titer at autopsy units/ml	
III	SS	190		230		40, 43[a]	
	DD	200		200		51, 60[a]	
	DS	220		270		25	
	DS+7		200		260		38
	DC	200		260		40	
	DC+7		190		210		45
	DCC	180		220		27	
	DCC+7		190		250		31
	DA	170		190		55	
	DA+7		150		200		60
	DAM	140		210		34	
	DAM+7		190		260		36
	DG	160		270		45	
	DG+7		150		200		52
	DGL	160		240		43	
	DGL+7		160		190		43
IV	DE	170		200		52	
	DE+7		140		160		46
	DEE+7		190		190		45
	DAM+7'		180		160		48
V	D1D	160		190		57	
	D8D	160		140		35	

[a]Second figure represents data for re-analysis of 6 samples at the same time as those of Experiment V.

Table 15. Values of "t" for comparisons of titers of complement, and properdin measured at autopsy, maximum titers of hemolysin, titers of hemolysin 10 days after injection of antigen, and estimated total hemolytic units produced within 14 days

Groups compared		Complement at autopsy	Hemolysin			Properdin at autopsy
			max. titer	10th day	total	
SS	DD	0.9	3.4**[a]	0.2	2.3*	1.3, 2.0[b]
DD	D1D	0.3	-	-	-	0.4[b]
DD	D8D	2.8*	-	-	-	2.0[b]
D1D	D8D	3.3**	1.2	0.8	-	1.6
DC	DC+7	-	0.8	0.2	0.4	0.5
DC	DCC	1.4	0.7	1.2	1.1	1.9
DC+7	DCC+7	1.4	1.4	1.2	1.4	2.1*
DCC	DCC+7	-	1.3	3.0**	2.3*	0.8
DA	DA+7	-	0.2	0.1	0.0	0.9
DA	DAM	0.7	0.0	0.4	0.2	2.6*
DA+7	DAM+7	1.4	1.8	3.1**	1.9	3.6**
DAM	DAM+7	-	1.2	2.0	1.2	0.2
DG	DG+7	-	0.4	0.0	1.0	0.7
DG	DGL	1.1	0.1	0.4	0.3	0.2
DG+7	DGL+7	2.0	0.4	0.4	0.5	1.4
DGL	DGL+7	-	0.1	0.1	0.2	0.0
DE	DE+7	-	0.8	0.2	-	0.9
DE+7	DEE+7	3.7**	1.1	0.5	-	0.2
DAM+7'	DE+7	0.6	0.6	0.9	-	0.2
DAM+7'	DEE+7	2.1*	0.4	0.4	-	0.3
DAM+7' (IV)	DAM+7 (III)	3.7**	0.0	1.1	-	1.3

[a]Probabilities of less than 0.05 indicated by (*); probabilities of less than 0.01, by (**).

[b]Calculated for data from re-analysis of part of groups in Experiment III.

Table 15. (Continued)

Groups compared		Complement at autopsy	Hemolysin			Properdin at autopsy
			max. titer	10th day	total	
SS	DS	0.8	2.2*	0.2	1.6	2.3*
SS	DS+7	0.7	2.3*	1.5	1.9	0.3
SS	DC	0.7	3.8**	0.8	2.6*	0.1
SS	DC+7	0.5	2.2*	0.4	1.5	0.6
SS	DCC	0.4	2.8*	0.1	1.5	2.0
SS	DCC+7	0.4	4.2**	2.1*	3.7**	1.2
SS	DA	1.1	1.4	0.9	0.3	1.7
SS	DA+7	1.0	1.5	1.1	0.3	2.5*
SS	DAM	0.4	1.3	0.5	0.5	0.7
SS	DAM+7	0.4	2.7*	1.5	1.8	0.5
SS	DG	0.9	2.7*	0.4	1.3	0.6
SS	DG+7	0.8	2.6*	0.4	2.3*	1.4
SS	DGL	0.1	2.1*	0.7	1.4	0.4
SS	DGL+7	0.9	2.6*	0.8	1.8	0.5
DD	DS	1.9	1.2	0.4	0.7	4.0**
DD	DS+7	1.7	0.7	0.5	0.0	1.7
DD	DC	1.9	0.2	0.7	0.0	1.1
DD	DC+7	0.5	0.9	0.3	0.4	0.7
DD	DCC	0.6	0.7	0.3	0.9	3.7**
DD	DCC+7	1.6	0.6	2.0	1.2	2.8*
DD	DA	0.3	2.0	1.1	1.9	0.4
DD	DA+7	0.0	2.5*	1.4	2.3*	1.2
DD	DAM	0.4	1.8	0.7	1.7	2.1*
DD	DAM+7	1.3	0.5	1.4	0.4	1.9
DD	DG	2.1	1.0	0.2	0.9	0.6
DD	DG+7	0.1	0.5	0.2	0.1	0.1
DD	DGL	1.3	0.6	0.6	0.4	1.0
DD	DGL+7	0.1	1.0	0.6	0.3	1.2

were similar in Experiments IIIb and IV, but individual groups varied considerably.

Complement activity increased slightly during protein repletion, but there were no significant differences in Experiment IIIb among titers at autopsy for groups which had been fed a single source of protein, or between those of repleted groups and stock or depletion controls (Table 15). However, in Experiment IV, titers were consistently higher in rats fed EE than in those fed E or AM. Values for groups fed egg protein relative to those for Group DAM+7' were approximately the same as for groups fed stock ration or casein diets relative to those for DAM+7; therefore, it seemed that egg protein may have been equivalent to stock ration and to casein for maintenance of complement.

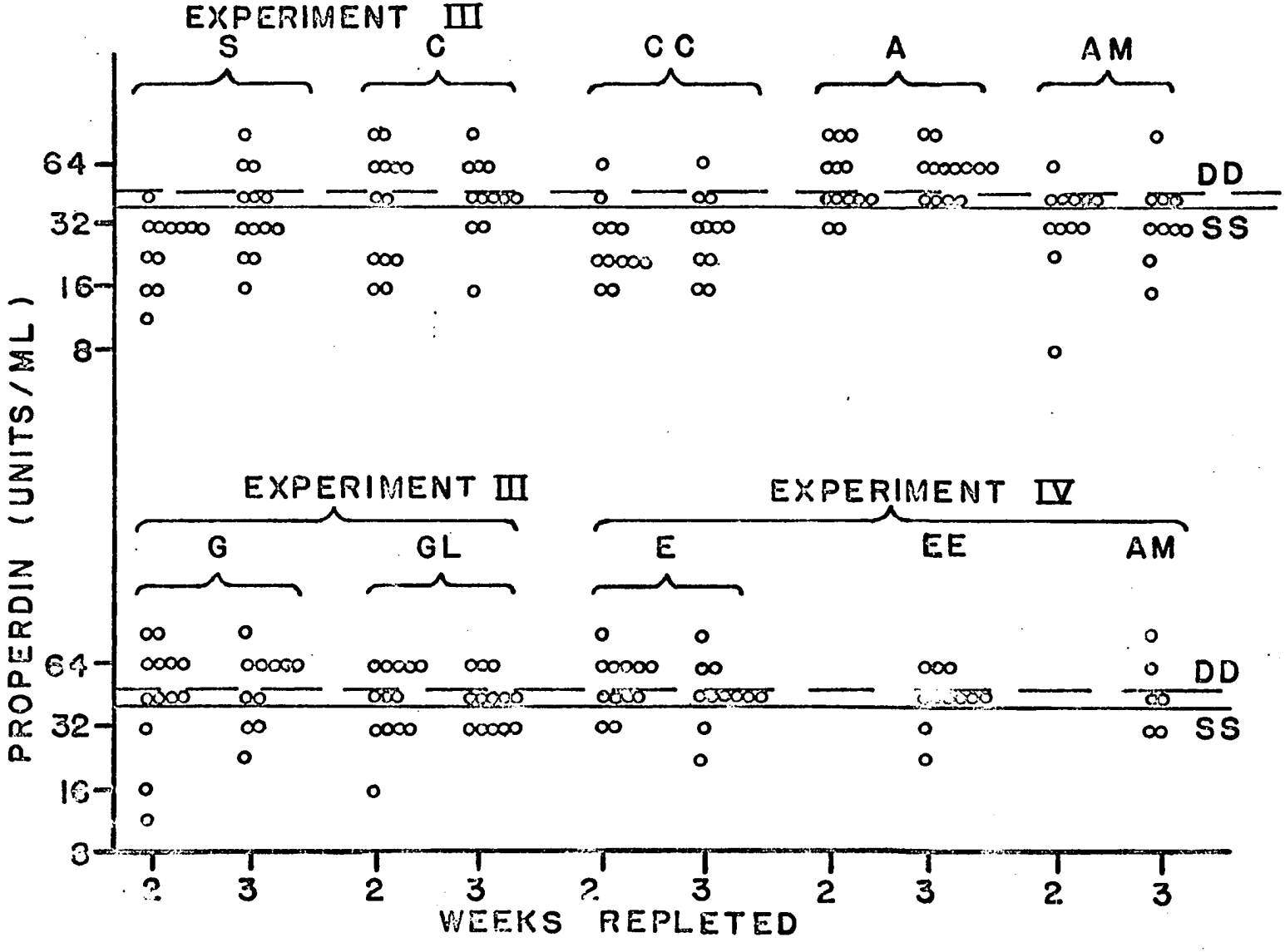
Complement titer was not related to concentration of hemoglobin or of total serum nitrogen after partial repletion; but in depleted animals, complement was lowest in animals which also had low concentrations of serum nitrogen.

Properdin

Mean titer of properdin after depletion for 10 weeks was only slightly lower than that of stock controls (Table 14). Because of small numbers of depleted animals for which data may be compared, the increased concentration after 3 or 6 weeks of depletion, over that of stock controls, was not of statistical significance (Table 15).

Titers of properdin in animals depleted for 4 weeks were unchanged by repletion with G, GL, or C for 2 or 3 weeks (Figure 6), whereas in rats repleted with S, CC, or AM, titers of properdin had decreased by the end of the second week. After the 3rd week, titers had risen significantly for

Figure 6. Titers of properdin in serum of individual animals after repletion for 2 or 3 weeks with various proteins, of stock controls and of animals which were depleted for 6 weeks


EXPERIMENT III
S
C
CC
A
AM
64
32
16
8
DD
SS
EXPERIMENT III
EXPERIMENT IV
G
GL
E
EE
AM
64
32
16
8
DD
SS
PROPERDIN (UNITS/ML)
2
3
2
3
2
3
2
3
2
3
WEEKS REPLETED

rats fed S, and slightly (but not significantly) for rats fed CC or AM. On the other hand, titers were elevated after 2 or 3 weeks of repletion with unsupplemented alpha protein (A). Thus, increasing the quantity of dietary casein from 9 to 18% or addition of methionine to alpha protein decreased the ability of rats to maintain concentrations of properdin, while gluten supplemented with lysine was no more or less effective than gluten alone. Titers of properdin were not significantly different among repleted groups in Experiment IV, in which only soy protein with methionine and egg protein were tested.

Assuming that total circulating hemoglobin remained constant, it was estimated that blood volume increased about 12 to 14% in 2 weeks of repletion. If rate of turnover of properdin was also constant, titers of about 50 units/ml at the beginning of repletion would have been diluted to about 44 units/ml, which was almost exactly the titer for rats fed only gluten or gluten with lysine as the source of nitrogen. This concentration exceeded that for rats repleted with S, CC, or AM.

Antibody formation

Hemolysin Maximum hemolysin titer in all groups in Experiment III correlated well with total hemolysin production, which was estimated from areas under curves constructed from log titers obtained at intervals over 2 weeks after immunization, assuming that measurable antibody production began 2 days after injection of antigen (Table 16). The length of time before attaining maximum values was not significantly affected by dietary treatment.

Table 16. Maximum titers of hemolysin and agglutinin and estimated total units of hemolysin produced within 14 days after immunization of rats injected with sheep erythrocytes on the 1st or 8th day of repletion with various proteins, of stock controls and of animals depleted for 1, 4, or 8 weeks before injection of antigen

Experiment	Group	Maximum hemolysin units/0.01 ml	Total hemolysin[a] log units/2 weeks	Maximum agglutinin units/0.025 ml
III	SS	80	330	33
	DD	33, 12[b]	187	14
	DS	45	222	19
	DS+7	40	188	19
	DC	34	185	20
	DC+7	42	209	22
	DCC	39	229	15
	DCC+7	28	145	23
	DA	55	306	29
	DA+7	57	308	24
	DAM	55	290	25
	DAM+7	38	207	20
	DG	42	237	21
	DG+7	38	180	24
	DGL	40	215	19
	DGL+7	42	205	18
IV	DE	23		
	DE+7	31		
	DEE+7	44		
	DAM+7'	38		
V	D1D	13		
	D8D	10		

[a]Calculated as area under curve of log titer vs. time, assuming antibody production began 2 days after immunization.

[b]Second figure for analyses repeated with samples from Experiment V.

In agreement with observations by Glabais (37), stock controls produced greatest maximum titers of hemolysin in response to injection of sheep erythrocytes (Figure 7). Magnitude of maximal response to antigen had decreased considerably after 1 week of protein depletion and had decreased further after 4 and 8 weeks (Table 16). Rats depleted for 4 weeks produced significantly less total hemolysin, as well as lower maximum titers than did stock controls, but titers for the 2 groups were not different on the 10th day after immunization (Table 15).

Repletion with stock diet did not increase immune response significantly above that of depleted animals; maximum titers were lower than for animals maintained on stock diet. In general, titers of hemolysin were higher in animals fed alpha protein than in those repleted with casein, gluten, or stock ration.

Although maximum titers did not differ significantly among groups fed diets similar in source of protein but differing in quantity of protein or in amino acid content, the stage of repletion at the time of immunization influenced antibody response of animals fed certain proteins. Thus, 10 days after injection, titers for animals repleted with CC or AM were lower if they had been injected on the 8th day of repletion than if they had been injected on the 1st day; on the other hand, repletion prior to immunization did not affect titers of rats repleted with C or A or diets containing egg protein or gluten.

Rats from Experiments IIIa and IVa were compared with those of Experiments IIIb and IVb to determine if differences among treatments were consistent at different times (Figure 8). Differences in titers between Experiments IIIa and IIIb may have resulted from variations in guinea pig serum

Figure 7. Titers of hemolysin in the serum after injection into rats of 1 ml 2% sheep erythrocytes on the 1st or 8th day of repletion with various proteins, after injection into stock controls and after injection into animals fed low nitrogen diet continuously

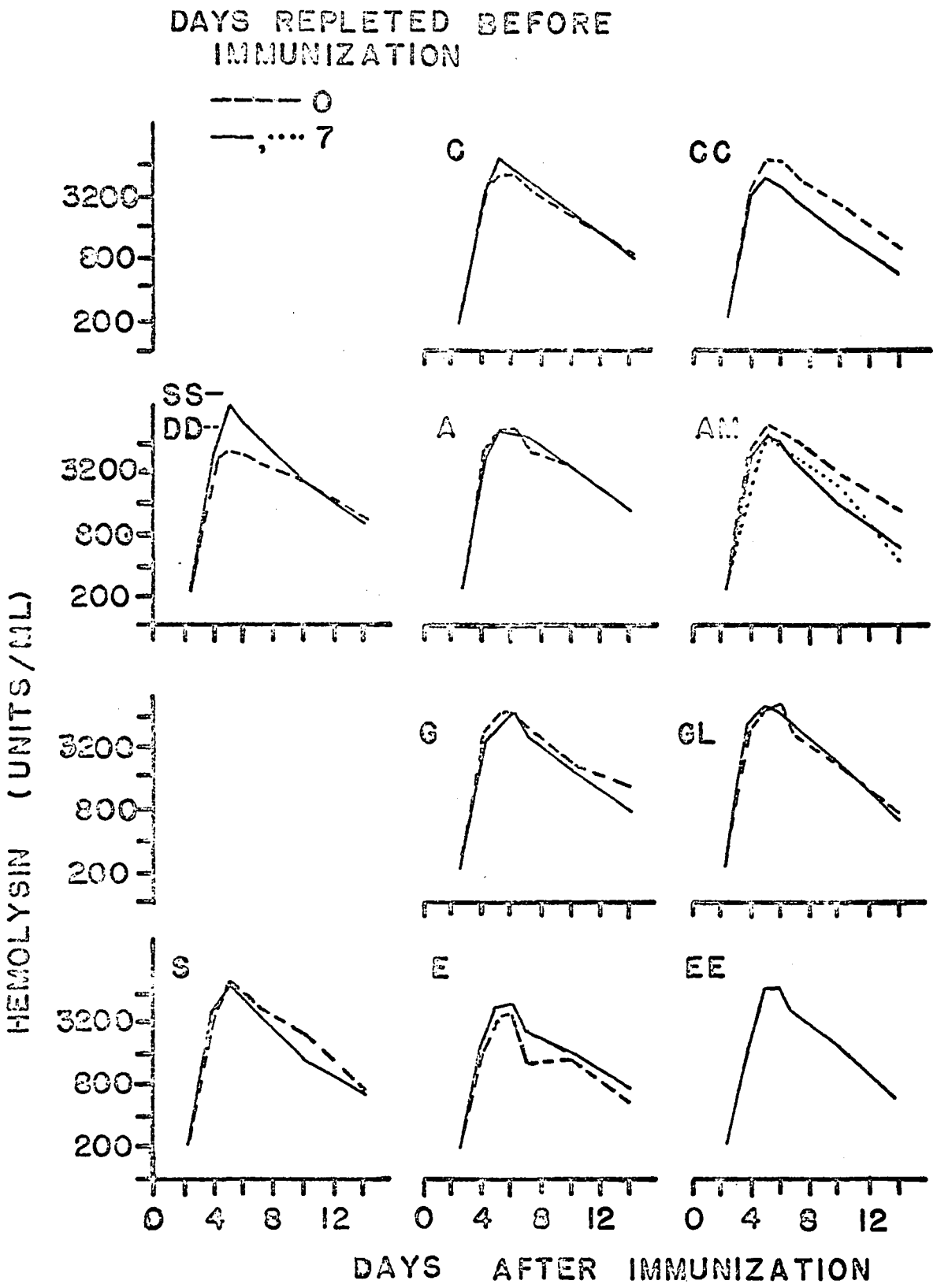
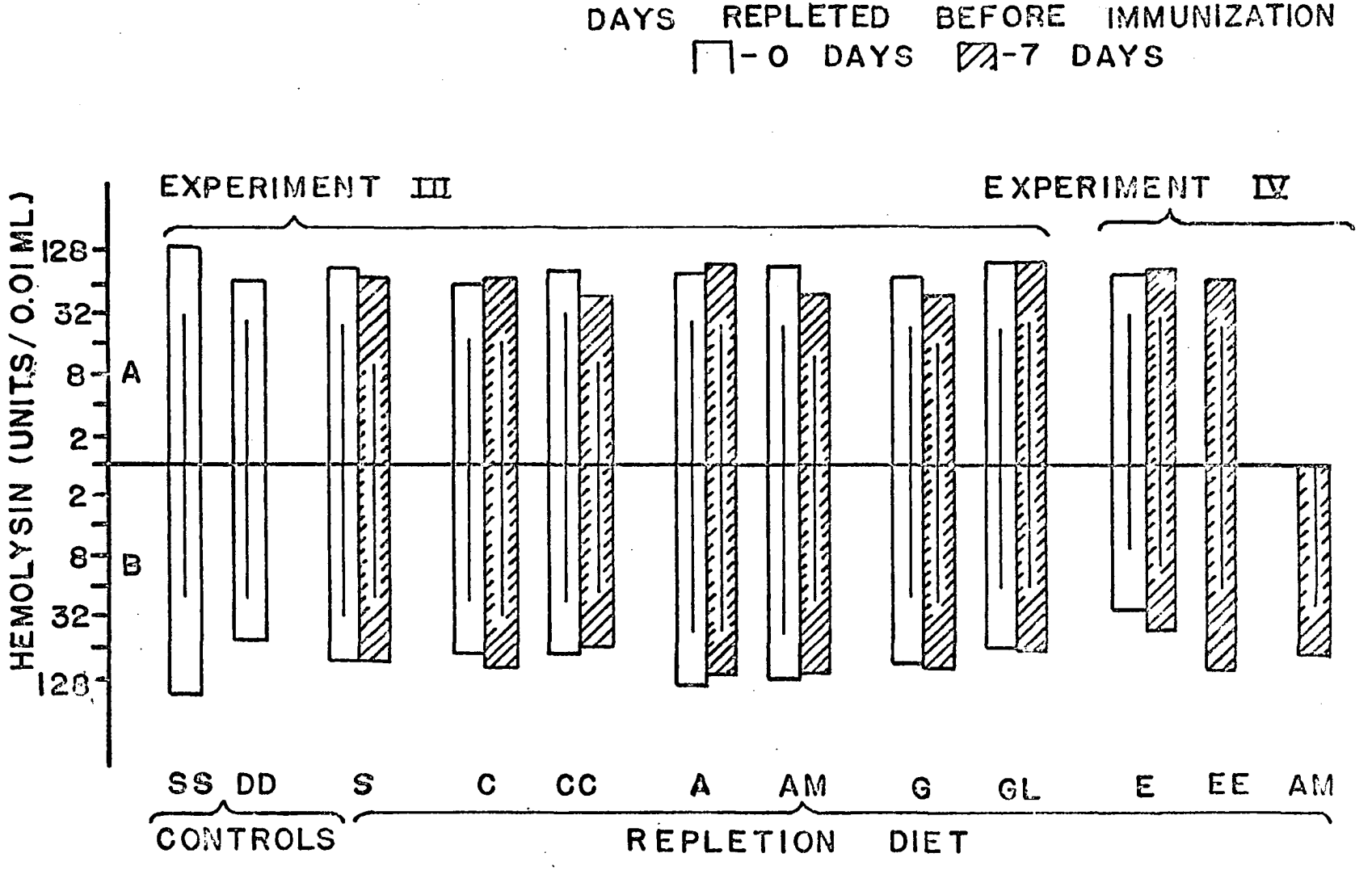

DAYS REPLETED BEFORE IMMUNIZATION
0
7
C
CC
SS-
DD--
A
AM
G
GL
S
E
EE
3200
800
200
HEMOLYSIN (UNITS/ML)
0 4 8 12
DAYS AFTER IMMUNIZATION

Figure 8. Comparison of maximum titers of hemolysin (bars) and titers 10 days after immunization (lines parallel to and within bars) in Experiments IIIa and IVa with those observed in Experiments IIIb and IVb

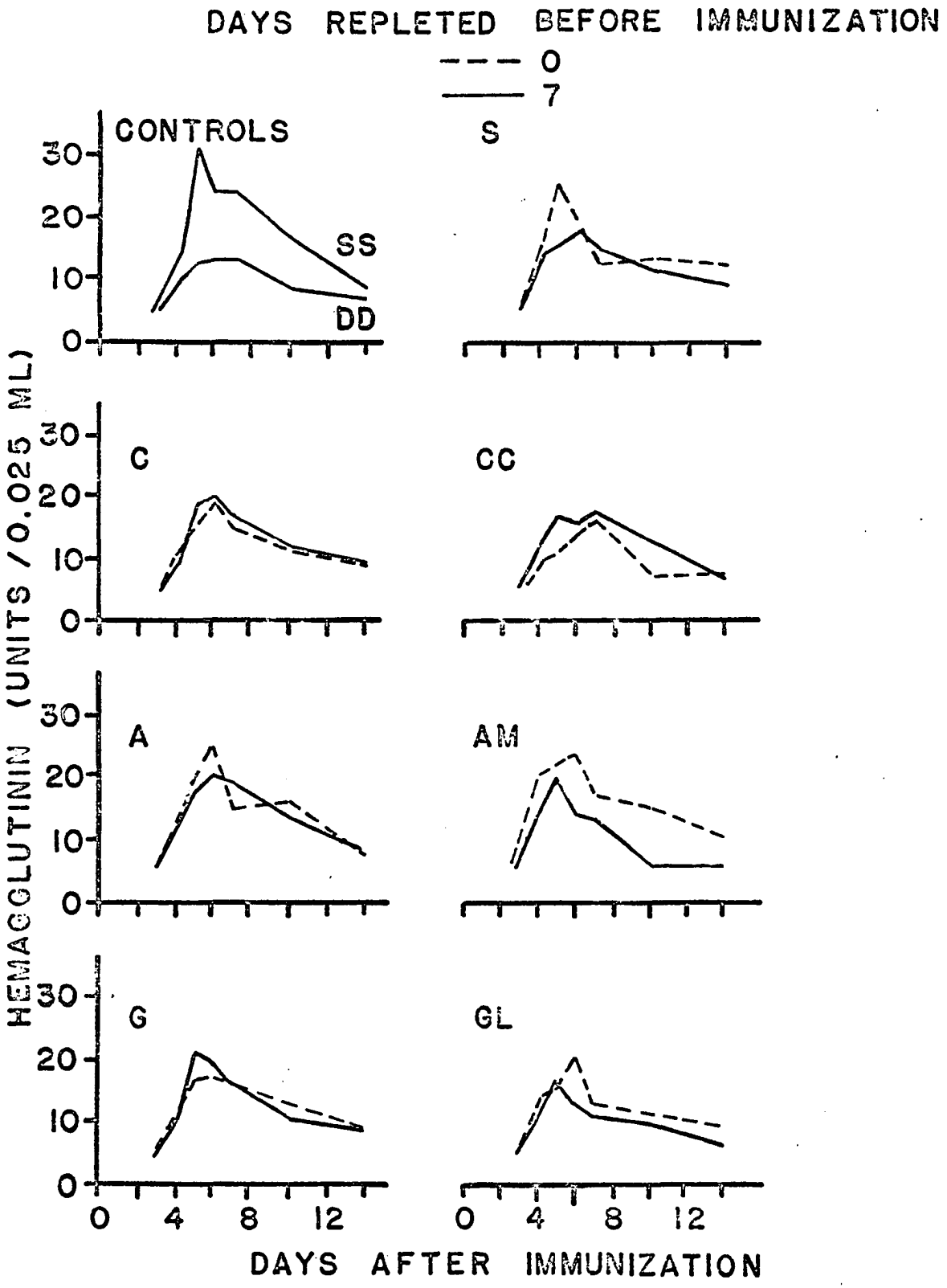

DAYS REPLETED BEFORE IMMUNIZATION
0 DAYS
7 DAYS
EXPERIMENT III
EXPERIMENT IV
HEMOLYSIN (UNITS/0.01 ML)
128
32
8
2
A
2
8
32
128
B
SS DD S C CC A AM G GL E EE AM
CONTROLS
REPLETION DIET

and blood cells used in the titrations. Since representatives of various dietary treatments were analyzed simultaneously, this factor should not have affected interpretation of data. In both sub-groups, maximum titers of hemolysin or titers after 10 days were less in groups fed AM and CC for a week before immunization than in animals fed the same diets beginning at the time of injection. Evidence of the efficacy of lysine supplementation of gluten or of increased concentration of egg protein, suggested in Experiments IIIa and IVb, was not supported by data from Experiments IIIb and IVa.

Hemolysin titers were positively correlated with concentration of complement in stock controls and in repleted animals of Experiments III and IV, except for those animals fed CC; for Groups DCC and DCC+7, the relationship of hemolysin to complement was reversed. In addition, decreased antibody titers observed after about 2 weeks of repletion with S, CC, and AM coincided with lowered properdin titers.

Agglutinin Stock control animals had higher agglutinin levels than depleted rats (Figure 9); values for repleted animals were generally intermediate. Although individual values for agglutinin were erratic, titers of hemolysin and agglutinin were significantly correlated. The one apparent exception was Group DCC+7; the mean agglutinin titer of this group was higher than for DCC, but the hemolysin titer was lower. Titers of agglutinating antibody were maximal on the 5th or 6th day after immunization. Since agglutinin and hemolysin curves in the present study generally were similar, and since sheep erythrocytes have been reported to be more effective as a stimulus for production of hemolysin than for formation of hemagglutinin by the rat (133), only sera from animals in Experiment IIIb were titrated for agglutinins.

Figure 9. Mean titers of hemagglutinin in the serum after injection into rats of sheep erythrocytes on the 1st or 8th day of repletion with various proteins, after injection into stock controls and after injection into animals fed low nitrogen diet continuously


DAYS REPLETED BEFORE IMMUNIZATION
0
7
CONTROLS
SS
DD
S
C
CC
A
AM
G
GL
HEMAGGLUTININ (UNITS / 0.025 ML)
30
20
10
0
0
4
8
12
DAYS AFTER IMMUNIZATION

DISCUSSION

Protein Depletion

Observed changes

Depletion of nitrogen following dietary deficiency of protein has been described in terms of weight loss or of net loss of nitrogen from the body. Attempts to assess more subtle changes than these in protein status have included measurements of nitrogen, nucleic acids, and enzymes of liver and proteins of serum (2, 134, 135, 136). Early stages of protein deprivation have been characterized in the liver by marked loss of weight, nitrogen, and ribonucleic acid (RNA) (137). Although loss of nitrogen in relation to either hepatic weight or deoxyribonucleic acid was maximal after 30 days, cytoplasmic protein of rat liver had decreased significantly after only 4 days of depletion (134). During depletion, activities of some enzymes of liver were reduced more rapidly than total nitrogen, while activities of others decreased at the same rate, and still others less rapidly than total nitrogen (138, 139, 140, 141, 142).

Not only were hepatic enzymes reduced, but concentration of serum albumin, formed by the liver, was also lowered by protein depletion (2, 143). A direct relationship of hepatic RNA to serum albumin, observed by Kawashima (135), implied that concentration of albumin indicated the overall extent of protein synthesis in the liver. Changes in concentration, rate of synthesis, and half-life of serum globulins, as well as of albumin, have been observed in protein depletion and repletion (Table 17). Jeffay and Winzler (144) observed increased half-life of albumin, but not of total globulin, in protein-depleted rats. However, in dogs, half-lives of 5 fractions of

Table 17. Effects[a] of protein depletion and repletion on concentrations and rates of synthesis and degradation of fractions of serum proteins in various species

Measurement	Species	Albumin	Alpha globulin	Beta globulin	Gamma globulin	Ref.
			Protein depletion			
Concentration	rat	D	D, D[b]	D	I	(1)
	rat	D	D	I	D	this study
	dog	D	I	-	I	(143)
	dog	D	I, D	D	NC	(145)
Rate of synthesis	rat	D	total globulin: NC			(144)
	rat	total protein: D				(146)
	dog	D	I, D	D	NC	(145)
Half-life	rat	I	total globulin: NC			(144)
	rat	total protein: I				(146)
	dog	I	I, I	I	I	(145)
			Protein repletion			
Concentration	rat	I	I, I	I	D	(1)
	rat	I	I	NC	NC or I	this study
	dog	I	I	I	I	(147)
	human	I	I, I	I	I	(2)
Rate of synthesis	human	I				(148, 149)
Half-life	human	D				(148, 149, 150)
	human	NC[c]				(151)

[a]D = decrease; I = increase; NC = no change.

[b]Alpha-1 and alpha-2 globulins measured separately.

[c]Changes in size of albumin pool not taken into consideration.

plasma protein all increased with the degree of depletion, though not at the same rates (145).

In the study reported here, adult rats fed no dietary protein lost body weight rapidly for 2 weeks, then at a slowly diminishing rate. Amounts of nitrogen in liver and in serum and concentrations of serum albumin and alpha globulin had decreased after 3 weeks of depletion; abilities to maintain concentrations of gamma globulin and to produce antibody to sheep cells were lessened by protein depletion for 4 to 6 weeks. Because of some unexplained differences between Experiments III and IV or V, it was not possible to conclude whether observed changes after different periods of depletion were due entirely to the degree of protein deficiency or to other factors.

Concentrations of hemoglobin appeared to be maintained for about 3 weeks after feeding the depletion diet, but then decreased. Protein depletion for 4 weeks did not significantly affect complement activity; however, after 10 weeks, complement titers appeared to be slightly lowered. Titers of properdin, a gamma-1 globulin (29, 87), were maintained for at least 6 weeks, although total concentration of gamma globulin had decreased by this time. Constancy of concentrations of some pre-existing globulins (properdin and complement) through more than 6 weeks of protein depletion suggested that depressed formation of hemolysin and agglutinin after depletion for only 4 weeks may have resulted from specific interference with activation of the antibody-forming mechanism. However, the length of time between injection of antigen and peak antibody titer was not affected by protein depletion. Properdin and complement apparently were not representative of globulins in general; therefore, measurement of major fractions of globulin represented only an average of proteins which did not behave similarly. In

contrast to data of Weimer (1), values for beta globulin in the present study increased as protein depletion progressed. This fraction of serum protein was the most difficult to measure, and the observed differences may be due in part to variation in defining the limits of this fraction.

Mechanisms of depletion

Different rates of depletion of various tissues have been attributed to differences in rates of turnover which were characteristic of individual proteins (152). Thus rat serum albumin with a half-life of 3.7 days decreased more rapidly in protein depletion than globulins, which had an average half-life of about 5 days (144, 153). Although a decrease in rate of synthesis and in size of the protein pool has been associated with decreased supply of amino acids, altered rates of degradation of various fractions of serum proteins in protein depletion tended to compensate for decreased rate of synthesis (145, 146).

Rate of turnover of a protein may be influenced by its site within the cell (154) and by the ease with which amino acids can enter the cell. Riggs (155) has published data suggesting that uptake of amino acids by tumor cells was a faster process than synthesis of proteins from the cytoplasmic amino acid pool.

Synthesis of specific proteins may be "induced" or "repressed" under various conditions. In higher animals substrate-induction of enzymes has been modified by adrenal cortical hormones, by such factors as oxygen tension or pH in the environment of the cell, and by diet, presumably through its effect on supplies of both substrate for the enzyme and of amino acids for synthesis of the enzyme (156, 157).

Enzyme induction has been related to regulation of synthesis of the enzyme through repressors. Jacob and Monod (158) suggested that both induction and repression of enzyme formation involved 2 genes, only one of which governed the sequence of amino acids of the enzyme. In induction, the second gene was thought to regulate rate of synthesis of the enzyme via some agent (perhaps RNA) which acted as an inhibitor unless it was bound to the inducer (substrate). Enzyme synthesis was thought to be repressed by analogous genetically-controlled repressors, which, acting in the opposite manner, inhibited enzyme formation only in the presence of some metabolite of a reaction sequence in which the repressible enzyme participated.

Induction of enzymes has been compared by Burnet (27) with the formation of antibody in response to antigen, but Haurowitz (30) has pointed out that antibody formation required the synthesis of a molecule which could be distinguished from any formed previously, whereas induction merely changed the concentration of a protein for which the cell already possessed genetically transmitted information.

Alterations in protein composition of a tissue during protein depletion may depend partly on pattern of amino acids available. The ability of cells to retain or to synthesize various amino acids, as well as the exogenous supply of amino acids, are among factors which determine the intracellular pattern of amino acids.

Protein Repletion

All body tissues are not repleted at the same rate; hence measurements of nitrogen retention and of body weight have only partially solved the problem of evaluating proteins for repletion. Some tissue proteins may be

depleted at the expense of others when dietary protein is inadequate for maintenance. One might suppose that the direction of the process regulating differential nitrogen loss would be reversed under conditions conducive to repletion of body protein. In the present study, repletion of carcass, of liver, and of serum, were measured to assess the value of proteins for repletion. Possible mechanisms of regulation of the repletion process will be discussed in relation to the data obtained.

Rates of turnover

Turnover of tissue proteins results from the concurrent but probably independently controlled processes of anabolism and catabolism (145). A change in either process can change the size of the protein pool; simultaneous changes of the same magnitude in both processes can alter the rate of turnover without changing the total amount of existing protein. Steinbock and Tarver (146) and Wannemacher et al. (145) have shown that both rate of replacement and half-life of serum proteins were altered in protein depletion and repletion. If, for example, the alteration in catabolic processes were a secondary, compensatory response to changes in rates of synthesis, as proposed by Gitlin et al. (151), a transition or lag period might exist between the time when the rate of synthesis was affected and the time when compensation in rate of degradation occurred. Existence of such a lag period in which one process predominated could account for the change in size of the protein pool which has been associated with a change in protein intake. Thus the overall pattern of repletion would depend upon the duration of the lag period in various tissues before attaining a new steady state.

Protein synthesis requires the proper assortment of amino acids, a source of energy, and microsomal ribonucleic acid (RNA), to dictate the

sequence of amino acids in the protein. A decrease in any of these can decrease the rate of synthesis; other modifiers of protein synthesis will be discussed later. Wannemacher et al. (145) suggested that RNA was protected from enzymatic breakdown by the presence of amino acids, and that destruction of RNA resulting from decreases in the amino acid pool was responsible for the slow rate of protein synthesis in protein deficiency.

Rates of degradation of tissue proteins change with protein status (145, 146), but attempts to measure half-life have led to some confusion, because of disagreement concerning validity of certain methods which have been used (148). The half-life of plasma albumin in infants decreased during repletion (148, 149), while a study of the plasma proteins in protein-depleted dogs (145) suggested that the magnitude of change in half-life may not have been the same for different fractions of protein.

In this study, nitrogen of liver and serum, which had decreased early in depletion and then stabilized, was repleted more rapidly than carcass nitrogen, loss of which had continued throughout protein depletion.

Titers of hemolysin, which were decreased in sera of depleted rats, generally were increased during repletion. In addition, repletion generally increased the titers of complement and altered the titers of properdin, although depletion for 4 to 6 weeks had not significantly affected either. Increase in complement titer appeared to be a general phenomenon of repletion, but was independent of changes in total serum nitrogen or body weight. Low titers of properdin and of hemolysin were observed at the end of 2 weeks of repletion with proteins which induced rapid weight gain and repletion of liver (Diets S, CC, AM). In contrast, animals fed Diet A had high titers of properdin and of antibody, even though they did not gain weight during

the repletion period. These findings suggested that rate of synthesis of properdin and hemolysin may have diminished when protein in carcass and in liver were being produced rapidly or that a general increase in rate of catabolism had occurred. Although maximum titers of hemolysin in rats fed DAM and DAM+7 were only slightly different, after 10 days the titer of Group DAM+7 was significantly lower than that of Group DAM. This could be interpreted as an early increase in rate of synthesis for both groups, with compensation in rate of degradation apparent in Group DAM+7 but not in Group DAM.

If one supposed that rates of anabolism varied widely, while rates of catabolism were more uniform among proteins, increased anabolism in carcass and liver or some concurrent change in the size of the amino acid pool, for example, might stimulate equally the breakdown of all proteins, including certain serum globulins even though their rates of anabolism had been affected little. The data here do not provide evidence for or against this possible sequence of events, but such a mechanism could account for many of the changes which were observed in protein depletion and repletion in this study.

Adaptation

The presence of substrates, or increases in their concentrations can induce synthesis of enzymes (156, 157). Decreased enzyme activity may result from reversible competitive feed-back inhibition by an end-product of a reaction sequence in which the enzyme participates, or from slowing the rate of enzyme synthesis by a genetically-controlled repressor which may be activated by some metabolic end-product (158, 159).

The extent to which induction, repression, and feed-back inhibition, as well as hormonal and other influences, control enzyme formation in multicellular organisms is not known (156, 159). However, during protein depletion of rats, early loss of hepatic enzymes involved in formation of urea (157) may have been due to decreased supply of substrate. Repletion governed solely by this process would be influenced by the needs for metabolizing dietary components. For example, a poorly utilized protein might increase enzymes which catalyze oxidative degradation of amino acids; whereas, to meet the requirement for energy for protein synthesis, an animal fed a protein of high biological value might tend to form enzymes for oxidation of carbohydrate and fat. Concentrations of enzymes of the succinic oxidase system of the liver and of arginase, transaminases, and other hepatic enzymes involved in amino acid metabolism responded to changes in protein nutriture (141, 156, 157), but a direct relationship was not always apparent (140, 142, 160). Although activities of these enzymes have sometimes responded to changes in quantity of potential substrate, induced by dietary manipulation, investigation of several metabolic pathways under various controlled conditions is needed to show the extent to which concentration of enzymes is a function of metabolic state, or the "need" for the enzyme.

Formation of serum albumin in protein repletion perhaps may be viewed as a variant of substrate-induction in which the substrate, non-esterified fatty acids, must be transported to fill a metabolic need for energy to support protein synthesis at another site in the body; this need may be reduced in protein depletion when protein synthesis is limited by lack of amino acids. On the other hand, an increase in alpha and beta lipoproteins has been associated with the lipemia of protein deficiency in some species

(136). Very fatty livers were observed in rats in this study after prolonged depletion, and beta globulins increased in depletion; unfortunately possible significance of data concerning lipid content of serum was not foreseen, and accumulation of this information was not included in the overall plan of the experiment.

The hypothesis that repletion occurred most rapidly in response to a specific metabolic need or stimulus was tested by measuring antibody formation. Sheep erythrocytes were injected as a standard stimulus with which to compare the capacities of animals to fabricate antibody. All animals attained peak antibody titers at the same time after injection, but the extent of synthesis and release of antibody and maintenance of titers were influenced by the kind of dietary protein and by the stage of repletion at the time of immunization. Effect of composition of dietary protein on antibody formation emphasizes that even when a strong, uniform stimulus to synthesis of a protein was present, the capacity to respond to the stimulus was modified by diet.

Amino acid balance

The amounts of amino acids in individual proteins, or tissues, are only grossly similar. Decreased supply of a single amino acid required in large amounts for a certain protein or tissue may promote synthesis of those proteins which contain little of that amino acid. Recent data by Block (161) suggested a possible way to test this hypothesis. Fourteen of the 18 amino acids measured in a large number of fractions of serum protein had relatively constant molar ratios to at least 2 other amino acids. Histidine and methionine were related to each other but not to any other amino acids; proline was not related to any other amino acids. Therefore, deficiency of

methionine, histidine, or proline might be expected to influence the composition of plasma proteins by a decrease in fractions containing a high proportion of the deficient amino acid. Concentrations of methionine and methionine plus cystine, based on Block's data, were higher in albumin and alpha, beta-2, and gamma-2 globulins than in gamma-1 globulins. In this study, animals fed alpha protein without methionine (A) generally formed more properdin, a γ-1 globulin (29), and more hemolysin than those fed a diet to which methionine was added (AM). Titers were also higher for rats fed C, which was low in sulfur-amino acids, than for those fed CC, which should have supplied adequate amounts (Table 18). Thus alteration of methionine (or methionine plus cystine) content of the diet influenced the quantities of properdin and hemolysin. In contrast to the apparent role of methionine and cystine in regulation of synthesis of properdin and hemolysin, lysine effected no similar changes; no differences in titers of properdin and hemolysin were noted between rats repleted with gluten plus lysine and those repleted with gluten alone. In rats fed egg protein which contained adequate amounts of lysine and of sulfur-amino acids, even as 9% of the diet, Glabais (37) observed that titers of anti-sheep hemolysin were higher when the diet contained 18% protein than when it contained 9%. Differences in groups fed egg protein in the present experiment were not statistically significant, but 18% protein tended to give higher titers than 9%.

Deficiencies of various amino acids in diets fed in this study (methionine-cystine in A, lysine in G, and threonine in GL) impaired growth and repletion of liver and serum. Effects of these 3 specific deficiencies were less easily distinguished from one another using these parameters as measures of biological value of protein than by measuring titers of properdin and

Table 18. Percentages of requirements of essential amino acids for repletion furnished by diets containing casein[a], soy alpha protein[b], wheat gluten[a], and egg protein[a]

Amino acid	Requirement[c] (mg/day)	Percent of requirement furnished by mean daily intake of diet							
		C	CC	A	AM	G	GL	E	EE
Lysine	132	102	195	62	82	18[d]	70	89	171
Histidine	48	100	194	65	85	58	67	81	154
Phenylalanine	102	91	176	59	78	75	83	95	183
Tryptophan	32	75	144	38	50	47	53	78	150
Methionine + cystine[e]	88	69	133	22	79	61	69	106	190
Threonine	98	72	139	48	63	38	43	86	164
Leucine	166	99	190	54	72	61	68	97	187
Isolencine	138	78	151	57	75	46	51	84	162
Valine	116	102	195	57	76	55	61	104	200

[a]Average of data compiled by Block and Weiss (162).

[b]Analysis of soy protein by General Biochemicals, Incorporated.

[c]Estimated for the 400 g protein-depleted rat, from data of Steffee et al. (163) for requirements for recovery of weight lost in depletion when rats were fed a diet furnishing 3.2 cal. and 0.1 g essential amino acids/g diet.

[d]Lowest percentage, if less than 100, is underlined.

[e]Assuming cystine could fill up to 80% of the methionine requirement (164).

hemolysin which were increased in deficiency of methionine-cystine, but not in other deficiencies. Threonine deficiency, which has been shown to stimulate fat synthesis and increase fat content of liver and carcass (165), was associated with fatty liver in rats fed Diet GL. Inability to distinguish effects of deficiency of lysine may have resulted from similarities of tissue proteins with regard to their content of this amino acid, or from dilution of dietary amino acids with amino acids from endogenous sources, which may have minimized imbalances of amino acids of the ingested protein.

SUMMARY

Titers of immunoproteins in sera of adult protein-depleted and -repleted rats and in stock controls were compared with data on body weight, composition of liver, and concentrations of nitrogen in serum and of various other proteins in blood. In order to follow the sequence of changes during protein depletion, some rats were fed a low nitrogen diet for 1, 4, or 8 weeks, injected with antigen, and fed the same diet for 2 additional weeks. Various dietary proteins were evaluated in repletion of other animals which had been depleted of protein for 4 weeks. Stock ration or a semi-synthetic diet containing either 9 or 18% casein (C, CC) or egg protein (E, EE), 9% soy alpha protein with or without 0.25% DL-methionine (AM, A), or 9% wheat gluten with or without 0.35% L-lysine (GL, G) was fed ad libitum during the repletion period. One milliliter of 2% sheep erythrocytes was injected on the 1st or 8th day of protein repletion. At the time of immunization, serum complement and hemoglobin were measured. Anti-sheep hemolysin and sometimes agglutinin were titrated 4, 5, 6, 7, 10, and 14 days after injection of antigen. Animals were sacrificed 2 weeks after immunization and analyses of fat and nitrogen in liver, hemoglobin in blood, and total nitrogen, protein fractions, complement, and properdin in serum were carried out.

After 3 weeks of depletion, the rate of weight loss had diminished, and total losses of nitrogen and of fat-free tissue of liver were almost as great as after 10 weeks. Concentrations of total serum nitrogen, serum albumin, and alpha globulin also had decreased significantly after 3 weeks, as had ability to form antibody to sheep cells; beta globulin had increased

slightly. After 6 weeks, concentrations of hemoglobin and of gamma globulin had decreased. Titers of complement and of properdin were not significantly altered until after 8 to 10 weeks of depletion. Excessive amounts of fat accumulated in the liver after 10 weeks of protein depletion.

Titers of complement in serum were variable and differences attributable to diet during repletion were difficult to evaluate. Concentrations of hemoglobin decreased after 1 or 2 weeks of repletion, then increased, but were not affected by the kind or amount of dietary protein. Fat-free liver and concentration of serum nitrogen tended to increase earliest in rats which were gaining weight rapidly. In general, weight of fat-free liver during repletion was more closely related to total serum nitrogen than to individual fractions of serum protein, which often did not change in proportion to weight.

Diets containing casein promoted rapid weight gain; but liver and serum nitrogen were repleted more rapidly with 18% casein than with 9%. Casein was utilized to a greater extent for synthesis of serum globulins than was alpha protein or gluten. However, titers of hemolysin in rats repleted with casein generally were not higher than in those fed other sources of protein. Titers of hemolysin and of properdin after 2 or 3 weeks of repletion were lower in rats fed 18% casein than in those fed 9%.

While soy alpha protein (A) did not allow gain in body weight, nitrogen in liver and serum increased slightly. Titers of both properdin and hemolysin were significantly higher when alpha protein was fed than when other sources of protein were fed. Supplementation of alpha protein with methionine (AM) caused moderate gain in weights of body and of liver and moderate increase in serum nitrogen; however, the titer of properdin de-

creased and the rate of fall of hemolysin titer increased, since 10 days after immunization rats which had been repleted for a week before injection of antigen had lower values than those injected at the beginning of repletion.

Animals fed wheat gluten (G) gained only small amounts of weight. Repletion of liver and of total serum nitrogen was slow, although concentrations of serum albumin appeared to be higher than when casein or alpha protein was fed. Addition of lysine to gluten (GL) increased slightly the rates of repletion of carcass, liver, and serum, but allowed accumulation of fat in liver. Gluten diets, compared with those containing alpha protein or casein, were intermediate with regard to antibody response; properdin titers remained unaltered in rats fed diets containing gluten.

Egg protein, which was tested in a separate experiment, was evaluated indirectly in relation to other dietary proteins by comparisons with animals fed Diet AM and with depleted animals studied simultaneously. Egg protein as 9% of the diet (E) was the most efficiently utilized source of nitrogen for weight gain, which almost equaled that of rats fed 18% egg protein (EE). Except that EE produced consistently higher titers of complement than did E, responses to the 2 diets differed little from each other. Egg protein generally appeared to be similar to casein except that no difference was observed in hemolysin titers of rats fed the 2 percentages, as occurred with diets containing similar quantities of casein.

Stock ration produced maximum rate of weight gain and repletion of liver and of serum nitrogen, but titers of properdin and of antibody were low after 2 weeks of repletion.

The processes of depletion and repletion of various body proteins were discussed in relation to 1) their rates of degradation and synthesis and possible effect on these rates of such factors as size of the amino acid pool, 2) adaptation or regulation of protein synthesis by specific stimulants (inducers) or inhibitors (repressors) of protein synthesis, and 3) the pattern of amino acids available for protein synthesis.

Changes in titers of specific immunoproteins (complement, hemolysin, and properdin) varied from each other during protein repletion and from the change in the proportion of the fraction of serum proteins to which the specific proteins belonged. Serum nitrogen and hepatic nitrogen responded more rapidly to protein depletion and repletion than did body weight or concentration of gamma globulin. Rapid weight gain was associated with lowered titers of hemolysin and properdin. Proteins low in sulfur-amino acids seemed to favor synthesis of properdin and hemolysin; for example, 9% alpha protein in the diet was associated with higher titers of properdin and hemolysin than was a lysine deficient diet containing 9% gluten, which prevented weight gain, without enhancing synthesis of these immunoproteins.

Measurements of specific immunoproteins, such as properdin or hemolysin, which could not be estimated by measurement of heterogeneous fractions of serum protein, potentially have wide application because they require only small amounts of serum for analysis and reflect changes in individual protein molecules. Extension of the present study to include variation in the concentrations of dietary protein to allow moderate rates of weight gain during repletion with various proteins might provide a clearer understanding of the metabolic changes which occur in protein repletion. Measurement of the activities of hepatic enzymes, also specific protein molecules, is re-

commended as a means of studying changes in metabolic patterns in an organ which is particularly sensitive to changes in protein nutriture, and may give additional insight into processes of protein repletion.

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APPENDIX

Table 19. Weight change and food intake in 4 weeks of protein depletion, and urinary nitrogen excretion during the 24th through 28th days of depletion of animals in Experiment III

Group	Number of rats	Weight initial g	Weight after depletion g	Weight change g	Weight change %	Food intake[a] g/28 days	Urinary nitrogen mg/day
SS	13	451±6[b]	524±9	+73±7	16.1	-	-
DD	14	461±9	359±8	-103±5	22.2	365±12	37.3
DS	12	460±8	360±8	-100±4	21.7	364±18	37.8
DS+7	13	448±8	357±9	-91±4	20.5	377±14	34.1
DC	13	461±9	360±7	-101±5	21.8	406±13	36.8
DC+7	12	453±6	362±7	-91±3	20.2	388±20	32.9
DCC	12	452±5	358±7	-94±4	20.8	374±20	34.7
DCC+7	12	457±9	363±9	-95±4	20.8	412±25	34.8
DA	13	469±14	369±10	-100±7	21.1	383±14	37.3
DA+7	13	459±9	359±9	-100±6	21.8	400±28	35.6
DAM	12	457±7	360±7	-97±4	21.2	402±19	36.0
DAM+7	13	455±9	358±7	-97±6	21.3	425±8	37.4
DG	13	475±14	366±10	-109±6	22.8	414±25	35.2
DG+7	12	447±6	362±5	-85±3	19.0	414±26	34.8
DGL	13	463±10	361±7	-102±8	21.9	376±9	41.6
DGL+7	13	457±11	363±6	-94±8	20.3	387±12	36.4

[a]Data for animals in Experiment IIIa only.

[b]Mean ± standard error.

Table 20. Initial age, weight change in 4 weeks of protein depletion, and food intake and urinary nitrogen excretion during the 24th through 28th days of depletion of animals in Experiments IV and V

Experiment	Group	Number of rats	Age[a]	Weight				Food intake	Urinary nitrogen[b]
				initial	after depletion	change			
			days	g	g	g	%	g/5 days	mg/day
IV	DE	12	107±3[c]	464±8	380±5	-83±7	18	85±2	36.4
	DE+7	12	100±3	448±8	369±7	-79±4	18	94±3	33.8
	DEE+7	11	100±2	444±4	364±5	-80±3	18	90±3	31.8
	DAM+7'	6	103±3	451±11	366±11	-86±7	19	84±6	-
V	D1D	6	126±6	483±16	-	-	-	-	-
	D8D	10	96±2	455±14	374±8	81±6	18	90±3	33.2

[a]At the beginning of the experiment.

[b]For animals in Experiments IVa and V only.

[c]Mean ± standard error.

Table 21. Microliters of serum containing one 50% unit of complement, measured in serum samples obtained from protein depleted and repleted rats and stock controls at immunization and at autopsy in Experiments IIIb, IV and V

Experiment	Group	Number of rats	Days repleted at immunization 0	Days repleted at immunization 7	Days repleted at autopsy 0	Days repleted at autopsy 14	Days repleted at autopsy 21
III	SS	4; 6	5.4±.8[a]		4.3±.6		
	DD	4; 6	5.1±.6		5.1±.6		
	DS	4; 6	4.5±.5			3.7±.4	
	DS+7	6; 6		5.0±.3			3.8±.4
	DC	5; 7	5.1±.6			3.8±.3	
	DC+7	6; 6		5.3±.6			4.7±.4
	DCC	5; 7	5.5±.6			4.6±.5	
	DCC+7	5; 6		5.4±.2			4.0±.3
	DA	5; 7	5.9±.7			5.4±.7	
	DA+7	6; 7		6.7±.3			5.1±.5
	DAM	4; 6	7.0±1.1			4.7±.7	
	DAM+7	6; 4		5.3±.4			3.9±.6
	DG	5; 7	6.1±.7			3.7±.3	
	DG+7	6; 6		6.7±.6			5.0±.6
	DGL	5; 7	6.3±.3			4.2±.3	
	DGL+7	7; 7		6.1±.4			5.2±.8
IV	DE	6; 12	5.8±.2			5.1±.4	
	DE+7	6; 12		7.2±.3			6.4±.2
	DEE+7	6; 11		5.3±.2			5.4±.2
	DAM+7'	6; 6		5.5±.4			6.2±.3
V	D1D	6; 6	6.1±1.1		5.3±.3		
	D8D	10; 10	6.4±.4		7.0±.4		

[a]Mean ± standard error.

Table 22. Coded titers[a] of hemolysin in protein depleted and repleted rats and in stock controls in Experiments III, IV, and V measured 4, 5, 6, 7, 10, and 14 days after injection of sheep erythrocytes

Experiment	Group	Days after injection of sheep erythrocytes					
		4	5	6	7	10	14
III	SS	11.8±.9[b] 12[c]	14.4±.6 13	13.2±.8 12	12.1±.5 13	9.4±.7 13	6.4±.5 13
	DD	11.0±.6 11	11.7±.6 14	11.4±.6 14	10.5±.6 12	9.2±.6 14	6.9±.6 14
	DS	10.6±.6 11	12.7±.6 11	12.0±.5 10	11.2±.4 10	9.5±.5 11	5.7±.5 12
	DS+7	10.9±.6 12	12.5±.7 13	11.5±.7 13	10.4±1.0 10	7.8±.8 12	5.4±.7 13
	DC	10.5±.5 12	11.6±.4 13	11.6±.4 12	10.5±.5 10	8.7±.5 12	6.2±.4 13
	DC+7	10.4±.7 8	12.6±.6 12	11.7±.8 11	11.0±.7 12	8.9±1.0 11	6.0±.9 12
	DCC	10.5±.8 11	12.3±.5 12	12.2±.5 11	11.5±.3 12	9.5±.4 11	6.6±.5 12
	DCC+7	10.1±.7 8	11.3±.5 12	10.9±.5 12	9.9±.3 11	7.6±.4 10	5.0±.4 12
	DA	11.7±.8 9	12.8±.6 12	12.9±.6 12	11.7±.6 11	10.3±.8 10	7.4±.7 13
	DA+7	11.2±.6 10	12.8±.5 12	12.8±.5 10	12.3±.4 13	10.2±.4 13	7.5±.4 13

[a]Logs of reciprocal titers x 2.

[b]Mean ± standard error.

[c]Number of samples analyzed.

Table 22. (Continued)

Experiment	Group	Days after injection of sheep erythrocytes					
		4	5	6	7	10	14
	DAM	11.6±.7 10	13.2±.5 12	12.8±.6 9	12.1±.8 11	9.9±.7 9	7.6±.7 12
	DAM+7	11.4±.6 8	12.5±.7 10	12.0±.5 10	10.8±.4 11	7.9±.7 9	5.0±.8 10
	DG	11.1±.6 12	12.1±.4 11	12.0±.5 12	11.3±.4 13	9.0±.6 12	7.5±.8 13
	DG+7	10.4±.6 10	11.5±.5 12	12.1±.6 11	10.5±.7 11	8.5±.7 11	5.9±.5 11
	DGL	10.8±.8 12	12.2±.8 12	12.7±.7 12	10.6±.8 13	8.5±.9 13	5.8±.9 13
	DGL+7	11.7±.5 9	12.5±.4 11	12.2±.6 12	11.2±.4 12	8.7±.6 12	5.5±.4 13
IV	DE	8.0±.6 5	10.5±.8 12	10.8±.9 12	7.7±1.7 6	7.8±1.1 12	5.0±.8 12
	DE+7	8.8±.7 6	11.3±.7 12	11.5±.5 12	9.7±.7 6	8.1±.7 12	5.8±.8 12
	DEE+7	8.2±.8 6	12.2±.8 11	12.4±.6 11	10.7±1.0 6	8.6±.8 11	5.1±1.0 11
	DAM+7'	-	12.2±.9 6	11.8±.7 6	-	9.2±1.0 6	4.2±.9 6
V	D1D	6.8±.3 4	9.2±.6 6	8.8±.7 6	7.8±.7 6	5.7±.6 6	3.2±.7 6
	DD[d]	-	9.2±1.0 6	8.8±.6 5	-	6.7±.5 7	-
	D8D	6.2±.7 6	8.1±.5 10	7.9±.4 10	6.8±.6 10	5.1±.5 10	2.8±.5 10

[d]Samples from DD of Experiment III analyzed again with those for Experiment V.

Table 23. Values of F for analyses of variance of weight gain, food intake, abdominal fat, concentrations of hemoglobin and complement at injection and at autopsy, and day of maximum hemolysin titer in Experiment III of 12 groups of rats which were repleted with diets containing casein, alpha protein, or gluten

Measurement	Source of variation	Degrees of freedom	F[a]
Weight gain in repletion	Kind of protein (P)	2	85.4**
	Supplementation[b] (S)	1	150.1**
	Time repleted (T)	1	19.4**
	Interactions: PS	2	12.2**
	PT	2	0.8
	ST	1	1.7
	PST	2	0.9
Total food intake in repletion	Kind of protein (P)	2	19.7**
	Supplementation (S)	1	26.0**
	Time repleted (T)	1	406.4**
	Interactions: PS	2	11.4**
	PT	2	0.6
	ST	1	0.4
	PST	2	3.1*
Abdominal fat	Kind of protein (P)	2	2.2
	Supplementation (S)	1	13.0**
	Time repleted (T)	1	3.5
	Interactions: PS	2	0.0
	PT	2	5.1**
	ST	1	0.8
	PST	2	1.8
Hemoglobin concentration at injection and at autopsy	Kind of protein (P)	2	0.7
	Supplementation (S)	1	1.1
	Time repleted (T)	3	60.6**
	Interactions: PS	2	1.1
	PT	6	1.2
	ST	3	2.0
	PST	6	1.3

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

[b]Addition of lysine to gluten, methionine to alpha protein, and of 9% casein to 9% casein were considered "supplements".

Table 23. (Continued)

Measurement	Source of variation	Degrees of freedom	F[a]
Complement titers at injection and at autopsy	Kind of protein (P)	2	4.0*
	Supplementation (S)	1	0.1
	Time repleted (T)	3	15.3**
	Interactions: PS	2	0.7
	PT	6	1.1
	ST	3	1.3
	PST	6	0.9
Days to maximum hemolysin titer	Treatments	11	1.0

Table 24. Coefficients of correlation for various proteins of blood and serum nitrogen in depleted and repleted animals and in stock controls of Experiments III and V

Comparison	Experiment	Groups	Degrees of freedom	Correlation coefficient[a]
Serum nitrogen at autopsy vs. Serum complement at autopsy	IIIb	all groups repleted 2 weeks with semi-synthetic diets	39	-0.02
		all groups repleted 3 weeks with semi-synthetic diets	34	+0.15
		SS	4	+0.09
		DD	4	+0.47
		DS	4	+0.82*
		DS+7	4	+0.43
	IIIb, V	D1D, D8D, DD	20	+0.63**
Serum nitrogen at autopsy vs. Hemoglobin at autopsy	III	all groups repleted 2 weeks with semi-synthetic diets	74	+0.58**
		all groups repleted 3 weeks with semi-synthetic diets	70	+0.17
Maximum hemolysin titer vs. Maximum agglutinin titer	IIIb	all groups	96	+0.49**
Maximum hemolysin titer vs. Total units of hemolysin in 14 days after immunization	III	all groups	181	+0.93**

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

Table 24. (Continued)

Comparison	Experiment	Groups	Degrees of freedom	Correlation coefficient[a]
Maximum hemolysin titer vs. Properdin titer at autopsy	III	all groups repleted 2 weeks with semi-synthetic diets	71	+0.08
		all groups repleted 3 weeks with semi-synthetic diets	68	+0.17
		SS	11	+0.49
		DD	12	-0.17
		DS	9	-0.30
		DS+7	11	-0.08

LIST OF ABBREVIATIONS

C'1, C'2, C'3, C'4	Components of complement
RP	Human serum after removal of properdin
R3	Human serum after removal of properdin and C'3
RNA	Ribonucleic acid

Diets

S	Stock ration
D	Low nitrogen depletion diet
C	9% casein
CC	18% casein
A	9% soy alpha protein
AM	9% soy alpha protein with methionine
G	9% wheat gluten
GL	9% wheat gluten with lysine
E	9% egg protein
EE	18% egg protein

Groups of Animals

SS	Stock controls; fed Diet S continuously
DD D1D D8D	Fed Diet D for 4, 1, or 8 weeks before and 2 weeks after immunization
DC DCC DA DAM DG DGL DE	Fed Diet D for 4 weeks before and Diet C, CC, A, AM, G, GL, or E for 2 weeks after immunization
DC+7 DCC+7 DA+7 DAM+7, DAM+7' DG+7 DGL+7 DE+7 DEE+7	Fed Diet D for 4 weeks followed by Diet C, CC, A, AM, G, GL, E, or EE for 1 week before and 2 weeks after immunization