Factors Affecting Human Antibody Response

I. Effects of Variations in Dietary Protein Upon the Antigenic Response of Men

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Although a great number of studies have been made of the antigen-antibody response, surprisingly little is known of its exact nature. In health, human tissues respond to a foreign protein by forming an antibody, usually a gamma globulin. In certain allergic conditions and perhaps some of the collagen-vascular diseases, antibodies themselves contribute to the abnormal state. Nature herself conducts experiments which give us valuable information. For instance, patients with agammaglobulinemia fail to respond to injections of bacterial or viral antigens.[1–4]

Other conditions which alter antibody synthesis have been recorded. Patients with hypoalbuminemia due to a variety of causes were found to respond poorly to the injection of typhoid antigens.[5] Similarly rabbits depleted of protein responded in the same way until protein was repleted.[6] However, an attempt to correlate the quantitative antibody response of patients to a pneumococcal polysaccharide with the initial concentration of protein in their serum failed completely. Four patients who had multiple myeloma responded poorly.[7,8] Additional studies in patients with blood dyscrasias showed faulty antibody responses.[9] Patients with untreated acute leukemia had exaggerated antibody responses while those with chronic lymphocytic leukemia had almost no response. These studies implicate the lymphocyte and the plasma cell in the process of antibody formation.

Certain kinds of therapy have been found to interfere with antibody production in man as well as in animals. These include x-ray, nitrogen mustard and other cytotoxic agents, and the adrenal steroids. Species differences become quite apparent in the steroid effect. For example, guinea pigs are most sensitive to cortisone whereas rats and human subjects are most resistant.[10–17]

Many studies in animals have indicated that deficiencies of certain vitamins, particularly pyridoxine and pantothenic acid, can impair antibody production.[18–25] Criticism has been expressed, however, regarding the reliability of antibody *titers* compared with *total* antibody nitrogen.[26] Balch found that malnourished patients had no defect in antibody production after immunization with diphtheria toxoid regardless of their level of serum proteins. Determination of total antibody nitrogen rather than the titer of antibodies was said to be a more reliable measure of the magnitude of antibody formation. Several workers disagreed with this opinion.

Other studies in man indicated that pyridoxine deficiency did not inhibit antibody

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TABLE I
Six Healthy Volunteer Subjects

Subject	Military Service	Age (yr.)	History and Physical Findings	Diet
1	None	40	Good health	High protein: egg yolk, milk solids
2	Army (9 yr.)	45	Good health	High protein: milk solids
3	Army (4 yr.)	43	Fractured skull 1939; good health since	Medium protein: egg yolk, milk solids
4	None	40	Cranial trauma 1958; good health otherwise	Medium protein: milk solids
5	Army (8 yr.)	43	Good health	Low protein: egg yolk
6	Army (3 yr.)	46	Good health	Low protein: milk solids

formation.[27] Slight depression of serum gamma globulin and unusually frequent infections were observed in human subjects made deficient in pantothenic acid.[28] No evidence of antibody inhibition was found, however, in patients with malignant disease who were fed a pantothenic acid-deficient diet.[29]

Inhibition of antibody production has been of interest to us not only from the academic standpoint, but also because it may provide additional means for studying diseases presumed to result from abnormal antibody formation by a person against his own tissues—the so-called autoimmune reaction.[30,31] In diseases such as systemic lupus erythematosus, nephritis, acquired hemolytic anemia and idiopathic thrombocytopenic purpura antibodies against the affected organs may occur.

Still another reason for investigation of the antigenic response of man is the hope that tissue transplantation some day may become feasible. Failure of homografts or of bone marrow transplantations to survive apparently results from the immunologic response elicited by such tissues.[32,33]

In a series of reports we have described our experiences in producing a deficiency of pantothenic acid in man.[34–36] Our own studies and those of others (*loc. cit.*) suggested several ways by which we might inhibit antibody production in man. Studies were planned to evaluate the effects of (1) dietary protein deficiency; (2) massive doses of exogenous gamma globulin; (3) pantothenic acid deficiency with and without the antagonist, omega methyl pantothenic acid; (4) pyridoxine deficiency; and (5) combined pantothenic acid and pyridoxine deficiencies upon the immunologic reactions of normal men. The methods employed in each phase of this investigation will be presented at the beginning of each section. The first study was designed to evaluate the effects upon antibody response of feeding each of three levels of protein: high, average and low.

METHODS

Part I: Dietary Protein

Six healthy volunteer subjects from the Iowa State Prison were hospitalized in the Metabolic Ward of the University Hospitals. The men ranged in age from forty to forty-five years. All were in good health (Table I). For four weeks routine meals were fed and initial examinations were made. Then each of the men was fed a different formula. They were divided into three pairs. The first pair of men received 20 gm. of protein per day, the second pair, 1 gm. per kg. of body weight and the third pair, 2 gm. per kg. of body weight (Table

TABLE II
Characteristics of the Diet

Subject	Diet	Protein (gm.)	Fat (gm.)	Calories	Nitrogen Balance
1	High egg, milk solids	143	111	2,492	+0.40
2	High milk solids	143	120	2,689	+0.23
3	Medium egg, milk solids	58	90	2,005	+0.22
4	Medium milk solids	72	132	2,935	−0.04
5	Low egg	20	132	2,908	−0.37
6	Low milk solids	20	111	2,480	−0.32

TABLE III
Source of Protein, Egg Yolk Diets

Subject	Total Protein (gm./day)	Protein Provided as	
		Egg Yolk (gm./day)	Nonfat Dry Milk Solids (gm./day)
1	143	340	232
3	58	138	94
5	20	119	0

II). Calories were adjusted by increasing or decreasing the fat and carbohydrate on the basis of food intake and weight changes during the preliminary period. One man of each pair received all the protein as nonfat dry milk solids. The second member of the pair received egg yolk or a mixture of egg yolk and dry milk solids as given in Table III.

The large amount of milk or eggs used in the diets providing 2 gm. of protein per kg. of body weight increased the intake of certain minerals above that of the diets with lower amounts of protein. To compensate for these increases, certain salts were added to equal the highest intake from food of the mineral. Vitamins were added in amounts to insure a generous intake of each. The range of intakes of vitamins and minerals provided by the food plus the additions are given in Table IV.

During the last three weeks of the experiment a general diet of vegetable food and nonfat dry milk solids was served.

Routine examinations of the patients included daily recordings of weight, temperature, pulse rate and any complaints they had. Aliquots of the dietary formulas were made. Accurate twenty-four hour collections of the urine were made. Stool was collected in seven day aliquots. In order to calculate balances, nitrogen was determined by the Kjeldahl procedure. Each week complete blood counts and urinalyses were made. Determinations of total serum nitrogen, erythrocyte sedimentation rate, hematocrit and serum electrophoresis (Spinco apparatus) were made each week. Every second week titers of typhoid H and O antibodies and tetanus antibodies were measured. Typhoid was determined by a standard agglutination technic.[37] Tetanus was determined by a mouse protective technic.[38] All men had been immunized against tetanus and typhoid either during military service or at the time of admission to prison. "Booster" injections of 0.5 cc. of both tetanus and typhoid antigens (commercial preparations) were administered at the beginning of the fifth, sixth and seventh weeks of the experimental period.

TABLE IV
Range of Intake of Minerals and Vitamins

Nutrient	Intake
Sodium (mg.)	2,750
Potassium (mg.)	5,702
Calcium (mg.)	4,992
Phosphorus (mg.)	4,312
Iron (mg.)	26
Magnesium (mg.)	499
Copper (mg.)	0.02–1.15
Sulfur (mg.)	403–1,320
Chlorine (mg.)	3,955–9,229
Iodine (mg.)	0.1
Niacin equivalents (mg.)	19–32.9
Ascorbic acid (mg.)	100–127
Vitamin D (I.U.)	449–520
Vitamin A (I.U.)	10,020–11,086
Thiamin (μg.)	1,306–1,850
Riboflavin (μg.)	1,920–7,411

RESULTS

The men accepted their formulas well and remained in good health. Except for two subjects who were fed low protein diets, the control weight was maintained. These two subjects lost 17 and 16 pounds and had a modest increase in erythrocyte sedimentation rate (44

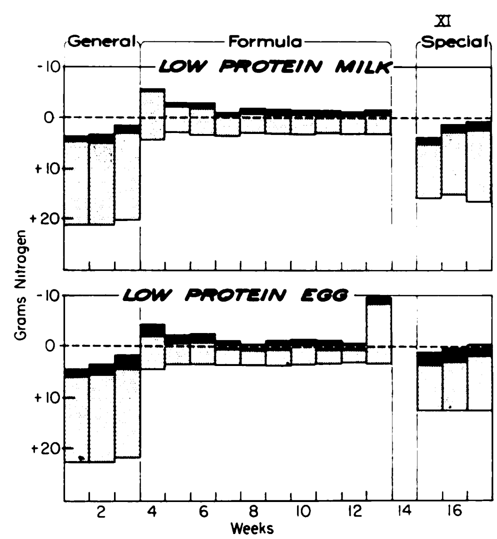

FIG. 1. Nitrogen balance studies of subjects fed a low protein formula.

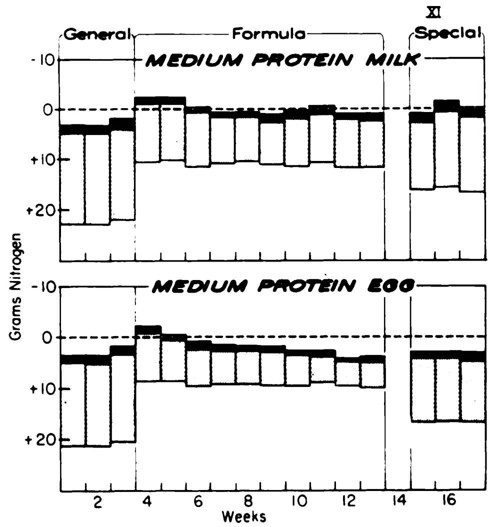

FIG. 2. Nitrogen balance studies of subjects fed an average protein formula.

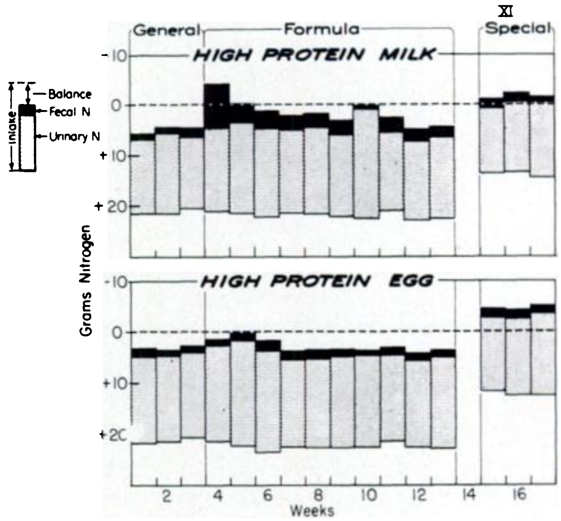

FIG. 3. Nitrogen balance studies of subjects fed a high protein formula.

and 32 mm. per hour, Westergren). One (subject 6) had a modest decrease in hematocrit (from 50 to 45 per cent) but no significant changes in red blood count or hemoglobin concentration. All these determinations were within normal limits for the remainder of the group as was the urine of all subjects. Nitrogen balances became negative only for the two men eating the low protein diets (Fig. 1, 2 and 3). Serum protein, nitrogen and the various fractions of protein showed no significant departures from normal for any subject.

Antibody titers against typhoid O and H antigens and tetanus toxin of all the men initially were substantial. The two men who were fed average amounts of protein had normal responses. Two notable findings were observed: (1) as the quantity of egg yolk was increased in the diet, the magnitude of antibody response decreased; and (2) in the one man fed a low protein milk diet the response was poor (Fig. 4).

COMMENTS

In view of the report of faulty antibody production in response to protein deficiency

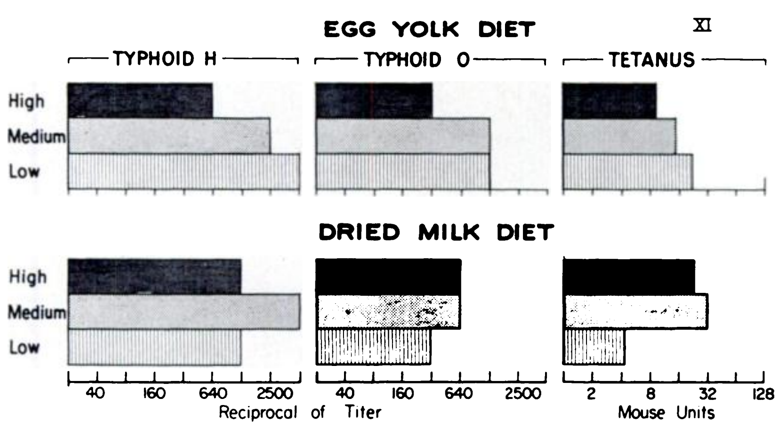

FIG. 4. Magnitude of antibody responses of the six men. Note that (1) as the amount of dietary egg yolk increased the antibody response decreased, and (2) in the man fed the "low milk" formula the response was poor.

both in animals and in man, we thought that in the two men fed low protein diets negative nitrogen balances might develop and that antibody formation might be less than in their colleagues. This was true of the man who received a low protein diet composed of dried milk solids, but was not true of his mate who ate the same quantity of protein in the form of egg yolk. This might be attributed to the "high biologic quality" of egg yolk proteins, even though the milk was supplemented with methionine.

A more surprising observation was that the magnitude of antibody response decreased as the quantity of egg yolk protein increased. Coburn reported that children fed egg yolks were relatively free from rheumatic fever. He suggested that egg yolk might contain something which affects antibody formation.[39] Our studies suggest that such a relationship may exist. Obviously, with such a small number of subjects, statistical evaluation is impossible. We are, however, planning studies to evaluate this factor.

SUMMARY

Six healthy men were fed diets with low, normal or high contents of protein. One man from each pair received egg yolk or egg yolk and skim milk solids as the sources of protein, the other man received skim milk solids only. The two men receiving the lowest quantity of protein were in negative nitrogen balance. Antibody responses to tetanus and typhoid antigens were poor in the man who ate a low protein diet composed of skim milk solids, but not in his colleague who ate egg yolk protein. As the quantity of egg yolk protein was increased in the diet, the magnitude of antibody response decreased.

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