Studies in Infantile Marasmus. IV. Impairment of Immunologic Responses in the Marasmic Pig

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Extract

We investigated the immunologic responses in an experimental model, the marasmic piglet. Two litters of miniature piglets of the Pitman Moore strain [46] were divided into two groups with five of the animals given normal diet and eight subjected to dietary restriction. The dietary intake of the experimental piglets was restricted to prevent further significant weight gain after the age of 35 days. The experimental animals displayed a well developed characteristic picture of severe undernutrition after 3 months of dietary restriction. Concentrations of immunoglobulins G and M (IgG and IgM) in both experimental and control pigs were similar. Antibody titers against erythrocyte A-antigen of experimental pigs were consistently lower (Table IV). Antibody responses after immunization to tetanus toxoid, sheep erythrocytes, and bacteriophage ϕ X 174 were remarkably similar in both groups (Tables V and VI). The experimental animals had a delay in appearance of IgG antibody after primary immunization with bacteriophage ϕ X 174. The physiologic lymphocytosis found in the control animals was absent in the experimental group. All lymphoid organs examined in the marasmic pigs displayed marked atrophy as compared to the control animals. No tissues were identified grossly as thymus in the neck or anterior mediastinum of the experimental animals. The germinal centers of the spleen and lymph nodes were poorly formed in the experimental animals. Delayed hypersensitivity to 2,4-dinitrofluorobenzene was demonstrated in only one of the five experimental animals while all four control animals demonstrated delayed hypersensitivity when tested at the same age. Four experimental pigs and three control pigs were immunized with BCG vaccine at 10 months of age. The mean diameter of tuberculin reaction of the experimental group was 6.5 mm; that of the controls was 27 mm when tested 4 weeks later. Both full thickness skin grafts and split thickness grafts underwent a delayed rejection in the experimental group. Lymphocyte transformation with phytohemagglutinin was studied at 6, 9, and 14 months of age in both control and experimental animals with similar results in both groups (Table VIII). Leukocytes from three experimental pigs had no increase in uptake of thymidine when tested in mixed leukocyte culture. Leukocytes from five experimental animals and three control pigs had normal leukocyte bactericidal activity against *Staphylococcus aureus* and *Serratia marcescens*. These studies provide presumptive evidence that marasmic pigs have defects in both humoral and cellular immunity. The deficiency in cellular immunity seemed greater and was manifest by thymic involution, lymphopenia, a decreased capacity to express delayed hypersensitivity, delayed homograft rejection, and absence of reactivity in the mixed leukocyte culture.

Speculation

Clinical experience and recent experimental studies indicate that malnutrition decreases resistance to infection. The results presented show that cell-mediated immunity is impaired in malnutrition. Much of the immunodeficiency in malnutrition may be mediated by hyperadrenocorticism.

Introduction

"Protein-calorie malnutrition coupled with infection is the greatest killer of infants and young children and the major cause of retarded child growth and development in today's world," stated the Committee on International Child Health in 1969 [11]. Infantile marasmus is one of the most common severe nutritional disorders affecting children of developing countries. Marasmus is the result of extreme and prolonged dietary restriction of proteins, calories, and other essential nutrients in early life. Marasmus is to be differentiated from kwashiorkor, which is the result of dietary protein deficits that are greater than dietary deficits in calories. Patients with kwashiorkor have edema and hypoproteinemia. In contrast, marasmic children have more nearly normal protein concentrations and no edema.

Clinical experience has indicated that malnourished children resist infections poorly, though the nature of the host deficiency is somewhat uncertain, no doubt because of the difficulty in conducting well controlled studies in humans [16, 19, 23, 31]. Children with protein-calorie malnutrition were reported to have atrophy of lymphoid tissue and a depression of cell-mediated immunity [38]. The studies reported here were designed to investigate the immunologic responses in an experimental model, the marasmic piglet.

Materials and Methods

Animals. Two litters of miniature piglets of the Pitman Moore strain [46] were used. The average weight at birth was 600 g. After weaning at 15 days, they were fed a commercial piglet diet [47]. The young animals reached an average weight of 4 kg at 35 days of age. The initial litter of nine piglets was divided into two groups with four of the animals serving as controls and five subjected to the experimental dietary restriction. Three additional piglets from a second litter were used as experimental animals, and one of the piglets from this litter served as a control animal. The animals were housed in individual cages. The diet of the exper-

imental piglets was adjusted to prevent further significant weight gain after the age of 35 days by restricting their dietary intake to 30 g of commercial pig diet offered three times daily. This limited dietary intake was continued throughout the entire study period. Water was supplied *ad libitum*. All animals were given 150 mg of iron by intramuscular injection of iron dextran on the 3rd day of life. All experimental procedures were performed in the temperature-controlled rooms in which the animals were housed.

Weights of four control and five experimental animals are depicted in Figure 1. The experimental animals displayed a well developed characteristic picture of severe undernutrition after 3 months of dietary restriction. There was absence of subcutaneous fat, marked muscle atrophy, and an increased amount of coarse body hair. The striking similarities of those animals to the clinical picture of infantile marasmus have been described in previous studies [30, 40].

Serum proteins. Serum protein concentrations and serum protein electrophoresis were performed by methods previously described [24, 28]. Serum concen-

Fig. 1. Weight of control and experimental pigs during the first 12 months of life. Diet restriction of the experimental animals was begun at 35 days of age.

trations of IgG and IgM were measured by single radial immunodiffusion method [29]. Antiserum to porcine IgM [48] and antiserum to porcine IgG [49] were obtained commercially. Immunologically pure porcine IgG [50] was used as the standard for IgG measurements. Purified porcine IgM was not available. The results of IgM determinations were expressed as percentages of a normal adult pig's serum [51].

Antigens and serum antibody determinations. Naturally occurring antibodies to human type A erythrocytes were determined by conventional hemagglutination technique. One milliliter of aluminum hydroxide-absorbed tetanus toxoid [52] was injected intramuscularly at the age of 50 days. Secondary immunization was carried out by giving a similar dose 6 months later. Antibody titers were determined by hemagglutination [39]. A saline suspension of 10^8 sheep erythrocytes (SRBC) per kg body weight in complete Freund's adjuvants [53] was given intramuscularly at the age of 1 year. Anti-SRBC antibody titers were determined by conventional hemagglutination.

The animals were immunized to bacteriophage ϕ X 174 by intravenous injection of 1.5×10^9 plaque-forming units (PFU) of phage per kg body weight. Three experimental piglets and three controls were immunized at the age of 80 days. A second group was primarily injected at 225 days of age. Secondary immunizations to phage ϕ X 174 were carried out 5 weeks later. Antibody activity was measured by phage neutralization and expressed as K-values or rates of phage inactivation [41]. Antibodies to bacteriophage ϕ X 174 were further characterized by reduction with 2-mercaptoethanol (2-ME) [18]. After the pigs were immunized with SRBC, selected serum samples were subjected to gel filtration on Sephadex G-200 [54] on 2.5×100 -cm columns using Tris buffer, pH 7.4. Eluates were collected in 6-ml fractions. The protein concentrations were determined by optical density measurement at 280μ .

Lymphocyte counts. The number of lymphocytes in the peripheral blood was calculated from differential counts of coverslip smears, and total leukocyte counts were performed using a hemocytometer. In one experimental and in one control animal the cisterna chyli were cannulated and lymphocyte counts were determined on the lymph obtained.

Sensitization with dinitrofluorobenzene. 2,4-Dinitrofluorobenzene (DNFB) [55] was diluted 1:10 in 90% acetone and 10% olive oil. The animals were sensitized by placing 1-cm squares of filter paper saturated with DNFB on the shaved skin of their backs. Delayed

hypersensitivity responses were elicited through intradermal injections of 0.1 ml of 1:1000 dilution of DNFB in the medial aspects of the legs.

Sensitization with BCG. BCG [56] was injected subcutaneously with complete Freund's adjuvant. Four weeks after the initial injection, intradermal injections of 0.1 ml mammalian tuberculin [57] were administered. Reactions to the tuberculin were measured 48 hr later.

Histopathology of peripheral lymph nodes, spleen, and thymus. Spleen and lymph nodes were obtained and thymuses were sought at postmortem examinations of three control and four experimental pigs. A peripheral lymph node was obtained from all other animals. The specimens were prepared for histologic examination.

Skin homograft. Two techniques of skin grafting were used. In the first, skin was taken from an unrelated donor pig and three full thickness homografts measuring 2 cm in diameter were placed on prepared beds in the backs of the experimental and control pigs. In addition, an autograft was placed on each animal. No sutures were taken. In the second technique, skin was again taken from an unrelated donor using a dermatome set to cut pieces of skin 10×5 cm and 0.02 mm in thickness. The skin was then cut into small pieces (1×1 cm). As many as 10 homografts were placed on a prepared bed of a single animal. Five autografts served as controls. The animals were anesthetized with intravenous phenobarbital. Second set grafts were not done.

Lymphocyte transformation with phytohemagglutinin (PHA). Blood was drawn in plastic syringes containing 100 units heparin per ml blood. Mononuclear cells were counted using a counting chamber. Aliquots of whole blood containing 10^6 mononuclear cells were placed in culture tubes containing 3 ml Eagle's TC medium [58] supplemented with 20% autologous pig serum, 500 units penicillin per ml, and 0.1 ml 1:10 dilution of PHA-M [59]. Total volumes were made up to 4 ml with TC medium. Control tubes were set up without PHA. These cultures were incubated for 72 hr at 37° . Eight hours before the end of the incubation period $4 \mu\text{Ci } ^3\text{H-thymidine}$ [60] were added to each tube. The cells were harvested by centrifuging the cultures at 1000 rpm for 10 min. The supernatant was then discarded, and the sediment was washed twice with 5 ml cold 0.2% NaCl. The cultures were centrifuged again, and, after the supernatant was discarded, 2 ml cold 5% trichloroacetic acid (TCA) were added to each tube. The tubes were then left over-

Table I. Total serum proteins and serum albumin concentrations of experimental and control pigs at 3, 6, and 12 months of age¹

Animal group	3 mos (55 days) ²	6 mos (146 days) ²	12 mos (330 days) ²
Total protein, g/100 ml			
Experimental (N = 7)	6.3 ± 0.31 ²	6.74 ± 0.23	6.10 ± 0.38
Control (N = 5)	6.4 ± 0.13	7.90 ± 0.47	7.36 ± 0.50
Albumin, g/100 ml			
Experimental (N = 7)	3.0 ± 0.34	3.01 ± 0.36	3.02 ± 0.21
Control (N = 5)	3.14 ± 0.25	4.27 ± 0.73	3.86 ± 0.27

¹ Values are expressed as means ± SD.

² Days on diet for experimental group.

Table II. IgG concentrations, in mg/100 ml, of experimental and control pigs¹

Age	Experimental	Control
2 days	2281 ± 788	2000 ± 993
1 month	542 ± 127	475 ± 194
3 months	1076 ± 165	1012 ± 125
6 months	1060 ± 155	1474 ± 223
12 months	1257 ± 91	1089 ± 133

¹ Values are expressed as means ± SD for 7 experimental and 5 control animals.

night at 4° and then centrifuged at 2000 rpm. The supernatant was discarded. DNA was extracted with 0.5 ml 5% TCA and heated at 95° twice for 10 min. Tubes were centrifuged once again, and 0.3 ml supernatant was placed in the vials containing 9 ml scintillation counting fluid made up of 4 g PPO [61] per liter toluene and 0.7 ml solubilizer [62]. Specimens were counted in a Beckman scintillation counter. Counts were expressed as number per 10⁵ cells. All culture tubes were set up in triplicate.

Mixed leukocyte culture. One-way mixed leukocyte cultures were carried out as described [1]. Leukocytes of a nonrelated donor were used to stimulate the responding cells. TCI 99 was buffered to a pH of 7.4 with "Hepes" (N-2-hydroxyethylpiperazine-N'-2-ethanesulfuric acid) [63]. Cultures were incubated for 72 hr and then pulsed with ³H-thymidine for 18 hr. The preparation of the material for counting was carried out essentially as described above.

Passive transfer of delayed hypersensitivity. A leukocyte suspension of cells from a highly sensitive DNFB-positive control was prepared as described above. Aliquots containing 5 × 10⁶ mononuclear cells were in-

jected subcutaneously into four experimental animals which had been shown to have lost DNFB hypersensitivity.

Leukocyte bactericidal activity. The bactericidal activity of leukocytes in peripheral blood was assayed as previously described [26].

Results

Serum proteins. Serum albumin concentrations of the experimental group were lower than controls at 6 and 12 months (Table I). IgG and IgM concentrations of both experimental and control pigs were similar (Tables II and III).

Serum antibody. Antibody against erythrocyte A-antigen first appeared in control animals at the age of 2 months and rose progressively in titer during the next 5 months. Anti-A titers of experimental pigs were consistently lower (Table IV).

Antibody responses after immunization to tetanus toxoid, SRBC, and bacteriophage ϕ X 174 were remarkably similar in both groups (Tables V and VI). The bacteriophage ϕ X 174 was cleared from the circulation by 1 week after immunization. All of the animals showed an increase in antibody titer after secondary immunization.

Table III. IgM concentrations of experimental and control pigs¹

Age	Experimental (N = 7), % adult serum	Control (N = 5), % adult serum
1 day	115 ± 46	115 ± 46
10 days	<10	<10
45 days	51 ± 7	52 ± 15
3 months	103 ± 22	102 ± 14
6 months	101 ± 16	102 ± 17

¹ Values are expressed as means ± SD.

Table IV. Reciprocals of natural anti-A hemagglutination titers of experimental and control pigs

Animal	Age, mos			
	2	3	5	7
C1 ¹	4	32	64	128
C2	16	32	32	64
C3	4	8	16	32
C4	8	32	64	128
E1 ²	0	2	4	8
E2	0	4	4	8
E3	2	4	16	32
E4	0	8	8	8
E5	8	8	8	8

¹ C1: Control animal 1.

² E1: Experimental animal 1.

Immunoglobulin class of antibodies. As shown in Table VI, most of the antibody obtained 1 week after immunization was sensitive to treatment with 2-ME. Four weeks after primary immunization most of the antibody from experimental animals was sensitive to 2-ME whereas antibody from controls was resistant. The experimental animals thus had a delay in appearance of IgG antibody after primary immunization. Both experimental and control groups made IgG antibody after secondary immunization.

Gel filtration of serum samples from two control

Table V. Reciprocals of antibody titers to tetanus toxoid and sheep red blood cells after primary and secondary immunizations

Animal	Tetanus toxoid		Sheep erythrocytes	
	4 weeks after primary	4 weeks after secondary	10 days after primary	10 days after secondary
C1 ¹	320	1280	512	2048
C2	1280	20480	128	1024
C3	80	320	128	1024
C4	1280		64	256
C5	320	5120	64	
E1 ²	80	1280	1024	2048
E2	80	5120	512	1024
E3	80	5120	256	1024
E4	1280	5120	128	512
E5	5210		128	512
E6	320		64	256

¹ C1: Control animal 1.

² E1: Experimental animal 1.

Table VI. Antibody response to bacteriophage ϕ X 174

Animal	Days on diet	Primary immunization		Secondary immunization ¹	
		1 week	4 weeks	1 week	4 weeks
C1 ²	50	7.9 ³ (<0.1) ⁴	1.4 (1.3)	26.2	16.3
C2	50	4.8 (<0.1)	0.58 (0.52)	ND ⁵	ND
C3	200	0.47 (<0.1)	13.3 (13)	ND	ND
C4	200	1.2 (0.12)	1.26 (1.12)	22.0	11.6
C5	50	2.9 (0.9)	0.15 (0.09)	ND	ND
E1 ⁶	200	7.1 (0.21)	16.1 (3.0)	ND	ND
E2	50	1.23	1.1 (0.2)	77.5	21.8
E3	200	0.3 (0.13)	1.3 (0.34)	13.8	16.0
E4	50	24.8 (0.1)	5.8 (0.82)	15.2	ND
E5	200	0.17	6.1 (0.78)	26.2	ND
E6	50	7.3 (0.24)	1.3 (0.56)	8.8	11.5

¹ Essentially no IgM antibody was detected after secondary immunization.

² C1: Control animal 1.

³ Neutralizing antibody expressed as K-values (see text).

⁴ Neutralizing antibody after treatment with 2-ME.

⁵ ND: not done.

⁶ E1: Experimental animal 1.

Fig. 2. Chromatography on Sephadex G-200 of a serum pool from control animals 10 days after primary immunization with SRBC. Tubes from the first and second protein peaks were pooled separately and tested for hemagglutinating activity. The bulk of the antibody detected by this method eluted in the first protein peak. Identical results were obtained in a similar study on a serum pool from experimental animals.

Fig. 3. Chromatography on Sephadex G-200 of a serum pool from control animals 25 days after primary immunization with SRBC. Tubes from the first and second protein peaks were pooled separately and tested for hemagglutinating activity. The bulk of the antibody detected by this method eluted in the second protein peak.

pigs taken 10 days after primary immunization with SRBC demonstrated that most of the antibody eluted in the first peak and was presumably IgM (Fig. 2). Serum samples obtained 25 days after primary immunization of control pigs with SRBC contained predominantly antibody eluting in the second peak, presumably IgG (Fig. 3). A pool of serum samples obtained from three experimental pigs taken 10 days after primary immunization with SRBC demonstrated a similar pattern to that shown in Figure 2. Most of the antibody eluted in the first protein peak. Serum samples obtained from the experimental animals 25 days after primary immunization demonstrated that most of

Fig. 4. Chromatography on Sephadex G-200 of a serum pool from experimental animals 25 days after primary immunization. In contrast with the findings in the control animals (Fig. 3), most of the antibody activity eluted in the first protein peak.

Fig. 5. Absolute lymphocyte counts of controls and experimental animals.

the antibody was still in the first peak and was presumably IgM (Fig. 4). These results are consistent with those obtained with 2-ME.

Lymphocyte counts. The lymphocyte counts from experimental and control pigs during the first 12 months of diet restriction are shown in Figure 5. The physiologic lymphocytosis found in the control animals was absent in the experimental group. Lymph was aspirated from the cisterna chyli in one experimental pig and one control pig at the age of 6 months. The experimental pig had 0.14×10^6 cells per mm^3 , and the control pig had 7.1×10^6 cells per mm^3 of lymph.

Morphology of lymphoid structures. All lymphoid organs examined in the marasmic pigs displayed marked atrophy as compared to the control animals. No tissues were identified grossly as thymus in the

neck or anterior mediastinum of the experimental animals. Histologic examination of the mediastinal connective tissue from experimental animals showed predominantly loose collagenous connective tissue. No adipose cells were seen. Focal aggregates of small spindle-shaped cells were found in the connective tissue elements. Some of these aggregates contained Hassall's corpuscles with keratinized cells. The spleen and lymph nodes of the experimental animals were smaller and hypocellular as compared to the controls. The germinal centers of the spleen and lymph nodes were poorly formed in the experimental animals. The splenic periarteriolar lymphoid tissue was more nearly normal in the experimental animals. Eosinophils were rarely seen.

Delayed hypersensitivity to DNFB and BCG. Five experimental pigs and four controls were sensitized with 2,4-dinitrofluorobenzene at 25 days of age. Delayed hypersensitivity was demonstrated in all experimental and control pigs 15 days later. Five experimental animals were retested after 46 days of diet restriction. Delayed hypersensitivity was demonstrated in only one of the five experimental animals while all four control animals demonstrated delayed hypersensitivity when tested at the same age. The animals were again tested at 126 days of age. None of the experimental animals expressed delayed hypersensitivity while three of the control animals reacted.

An attempt was made to transfer passively delayed hypersensitivity to DNFB in the four experimental animals at 128 days of age. Twenty-four hours after injection of a leukocyte suspension from a sensitized animal, four of the four injected experimental animals expressed delayed hypersensitivity to DNFB.

Four experimental pigs and three control pigs were immunized with BCG vaccine at 10 months of age. All were injected with tuberculin 4 weeks later. The mean diameter of induration of the experimental group was 6.5 mm; that of the controls was 27 mm. The main thickness of induration was 2 mm in the experimental group and 6 mm in the control group.

Skin homografts. Results of skin homograft studies in experimental control animals are shown in Table VII. Both full thickness grafts and split thickness grafts underwent a delayed rejection in the experimental group. The full thickness grafts were applied at 10 months of age and the split thickness grafts at 12 months of age.

Lymphocyte transformation. Lymphocyte transformation with phytohemagglutinin was studied at 6, 9, and 14 months of age in both control and experimen-

Table VII. First set skin homograft rejection in experimental and control pigs

Group	Method (No. of animals)	No. of skin grafts	Graft rejection, days	
			Median	Range
Control	Full thickness (N = 3)	9	7	5-9
	Split thickness (N = 1)	10	8	7-10
Experimental	Full thickness (N = 6)	18	13	7-19
	Split thickness (N = 2)	20	15	12-18

Table VIII. Tritiated thymidine incorporation of lymphocytes stimulated with phytohemagglutinin in 9-month-old experimental and control pigs¹

Animal	Counts/min/10 ⁵ mononuclear cells ²
C1 ³	46,877 ± 11,590
C2	50,674 ± 16,800
C3	61,371 ± 16,700
E1 ⁴	39,520 ± 3,514
E2	58,066 ± 5,049
E3	55,709 ± 10,271
E4	52,421 ± 10,298
E5	69,206 ± 23,900

¹ Control cultures of unstimulated lymphocytes all had less than 2000 counts per minute per 10⁵ mononuclear cells.

² Each value is a mean ± sd of 3 replicate tubes.

³ C1: Control animal 1.

⁴ E1: Experimental animal 1.

tal animals with similar results in both groups (Table VIII). Results of mixed leukocyte cultures at 14 months of age are shown in Figure 6. Leukocytes were obtained from three experimental pigs, two control pigs, and one nonrelated donor pig. The results are presented as thymidine incorporation of stimulated and nonstimulated leukocytes in culture. It will be observed that the stimulated donor and control leukocytes incorporated significantly increased amounts of thymidine, whereas leukocytes from three experimental pigs had no increase in uptake of thymidine when mixed with donor leukocytes.

Leukocyte bactericidal activity. Leukocytes from five experimental animals and three control pigs were tested for leukocyte bactericidal activity with *Staphylococcus aureus* and *Serratia marcescens*. The tests were performed at 3, 6, and 12 months of age. Leukocytes from both the experimental and control animals killed the bacteria at a rate comparable to that seen in healthy adult humans.

Discussion

These studies provide presumptive evidence that marasmic pigs have defects in both humoral and cellular immunity. The deficiency in humoral immunity was apparent as a delayed appearance of IgG antibody after immunization with bacteriophage ϕ X 174 and sheep erythrocytes and low titers of natural anti-A antibodies. The low titers of anti-A antibodies may be due in part to the limited antigenic content of the restricted diet. The deficiency in cellular immunity seemed greater and was manifest by thymic involution, lymphopenia, a decreased capacity to express delayed hypersensitivity, delayed homograft rejection, and absence of reactivity in the mixed leukocyte culture.

The findings complement those of clinical studies on infections and malnutrition. Severe malnutrition is largely a problem of infants and preschool children in underdeveloped countries. Infections and malnutrition most often act synergistically in this age group. The metabolic demands of infection often accentuate the manifestations of nutritional deficiency, and common infectious diseases are more severe in malnourished children [37]. The case fatality rate of measles in Guatemalan villages was 11.5% in infants and 4.5% in all age groups [17]. The peak mortality rate was in the 2nd year of life. Whooping cough, mumps, chicken pox, and rubella were also more severe in malnourished children [36].

Fig. 6. One-way mixed leukocyte culture of cells from an unrelated donor pig (D2), two control pigs (C3 and C5), and three experimental pigs (E2, E6, E7). Stimulating cells from a second unrelated donor pig (D1) were treated with mytomycin C to prevent incorporation of ³H-thymidine when compared with results in unstimulated cultures.

There are few reports of clinical studies on humoral immunity in malnutrition. Total serum proteins and serum immunoglobulins were normal in marasmic children [9], except that IgM concentrations were elevated [32]. Antibody responses in marasmic children have apparently not been investigated. In kwashiorkor, both normal and decreased antibody responses were found [4, 6, 14, 33, 34].

Cellular immunity seemingly was not investigated systematically in malnourished children until recently. Isolated observations were made of lymphopenia [8], thymic and lymphoid hypoplasia at autopsy [43], and diminution in the tuberculin reaction in tuberculous children [20] and after BCG vaccination [21]. Passive transfer of delayed hypersensitivity to tuberculin was effective in one study [5].

In a study of black African children with protein-calorie malnutrition, Smythe *et al.* [38] found lymphoid hypoplasia, lymphopenia, decreased capacity to express delayed hypersensitivity, and impaired transformation of lymphocytes with phytohemagglutinin. Our results and those of Smythe *et al.* complement each other in concluding that protein-calorie malnutrition produces a deficiency of cell-mediated immunity.

Malnutrition has been reported to enhance and also to diminish resistance of experimental animals to infection [37]. As pointed out by Woodruff and Kilbourne, most of these studies were done prior to 1950, and investigators were necessarily limited in the techniques available for study. In a careful analysis, malnutrition was found to increase the susceptibility of mice to Cocksackie B virus infections. Malnourished mice had an increased tissue concentration of virus, gross cardiac lesions, hepatic lesions, and increased mortality [45].

Woodruff also found that malnourished mice had lymphoid hypoplasia, lymphopenia, decreased early antibody response, and decreased interferon production. Feeding of a normal diet for 40 days restored the normal resistance to viral infections and normal appearance of lymphoid tissue [44].

The findings reported here in marasmic pigs, the experimental studies of Woodruff, and clinical observation all support the concept that malnutrition allows common infections to exhibit greater severity by decreasing host immunologic responses. Both humoral and cellular immunity seem to be affected though the defects in cellular immunity may be more significant. Defects in other mechanisms of host resistance, such as interferon, may also be important.

The immunologic impairment of malnutrition may

be mediated, in part, by hyperfunction of the adrenal cortex. Starvation is known to cause adrenal hyperplasia [2]. Plasma cortisol levels were high in marasmic children [25]. Corticosteroids are capable of producing most of the changes of immunologic function seen in marasmus, including lymphopenia, thymic atrophy, lymphoid hypoplasia, impaired antibody response, and defective cellular immunity [13, 42].

Cohen and Claman [10] recently presented evidence that the corticosteroid-sensitive phase of the mouse antibody response to sheep red blood cells was the bone marrow-derived precursor of antibody-forming cells. The influence of corticosteroids on immunologic function is complex and requires further investigation.

Adrenal hormones may not mediate all of the effects of malnutrition on immunity. Woodruff [45] found that adrenalectomy of starved animals only partially corrected the abnormality in absolute lymphocyte counts.

The delay in appearance of IgG antibody in marasmic pigs is apparently not caused by a failure in maturation of the pigs, since Kim *et al.* [27] have demonstrated that the young piglet has the same antibody response as the mature animal.

The present study provides another example of the relative resistance of the IgM-producing cells to insult. IgG antibody response is also suppressed to a relatively greater extent than IgM antibody response by immunosuppressive drugs [12, 35], antilymphocyte serum [15], and irradiation [22].

The thymic involution and lymphoid hypoplasia in the marasmic pigs were similar to those reported in malnourished animals [2] and man [43]. There was a striking depletion of lymphocytes, lymphoid follicles, and germinal centers in the lymph nodes of the marasmic pigs. Similar changes have been produced by administration of large doses of corticosteroids [3].

Divergent results were obtained in studies on lymphocyte transformations with phytohemagglutinin and in the mixed leukocyte culture. Lymphocytes from marasmic pigs functioned normally in the test with phytohemagglutinin but failed to react in the mixed leukocyte culture. A similar dissociation of lymphocyte transformation with phytohemagglutinin and the mixed leukocyte culture was observed in adult thymectomized rats treated with antilymphocyte serum [7]. This dissociation may be due to a quantitative deficiency in normally functioning thymus-derived lymphocytes. Relatively more normal thymus-derived lymphocytes may be necessary for a normal response in the

mixed leukocyte culture than in the transformation reaction with phytohemagglutinin.

Malnutrition is one of the most important health problems in the world, and infection constitutes one of the major causes of morbidity and mortality. Much remains to be learned concerning the interactions between nutrition, infections, and host resistance. Before the interaction between infection and malnutrition can be understood, further investigations will be required on immunologic function in malnourished children, immunologic function in malnourished animals, and resistance of malnourished animals to experimental infections.

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