THE EFFECT OF MALNUTRITION ON THE INFLAMMATORY RESPONSE

As Exhibited by the Granuloma Pouch of the Rat*

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Numerous field and clinical observations and the results of many experimental studies have shown that nutritional deficiencies and infections in both human beings and experimental animals may be interrelated (1–4). Often, there appears to be a heightened susceptibility to infection on the part of the host (5–10). No complete explanation for this is available.

The present investigation was designed to determine what effect malnutrition, induced by feeding a protein-deficient diet, has on the inflammatory response as exhibited by the granuloma pouch of the rat (11), and to study the behavior of some of the adaptive mechanisms accompanying malnutrition and inflammation. Severe protein malnutrition has been shown to depress the reticuloendothelial lymphoid system (12), and to result in a decrease in the number and function of cells involved in antibody production and phagocytic activity (12–16). Also, nutritional deficiency may seriously impair the latter or the repair stage of the inflammatory response (17–20). However, little is known of the effect of malnutrition on the over-all process of inflammation. This was studied in the present experiments by means of the granuloma pouch technique of Selye (11) which was selected for use because:

(a) Pouch formation places a limited amount of stress on the host (21).

(b) The pouch provides a semi-independent body cavity of a reproducible size and structure (11). Unlike natural body cavities such as the peritoneal cavity, the injection of irritants into the pouch cavity does not directly affect other body organs, e.g., liver, kidney, and spleen.

(c) The degree of inflammation can be appraised by the weight and thickness of the pouch wall, and the volume of exudate (11, 22).

(d) The chemical (21) and cellular composition of the various components of the pouch can also be studied.

Materials and Methods

Two experiments were performed. In Experiment A, a protein-deficient diet was fed to Sprague-Dawley rats 2 wk before the preparation of the pouch, and in Experiment B, the deficient diet was given to Wistar rats 1 day after the pouch was prepared. The local and systemic reactions called forth by the production of the inflammatory lesion in the above animals were compared with the reactions exhibited by animals fed a diet containing adequate protein.

Diets.—Diets and water were given ad lib. Rats on the poor diet consumed less than those given the complete diet. However, neither pair-feeding nor force-feeding techniques were employed because of the additional stress factors that would be involved. The low protein corn diet described below was chosen because its amino acid deficiencies were similar to those in the diets of malnourished children in Central America where this study was undertaken. Ad lib. feeding with the mixed diets was thought to parallel more closely the situation found under normal conditions in humans.

Complete diet: This was estimated to contain about 28% protein, and was made up of 500 g ground yellow corn, 30 g Hegsted (23) mineral mixture,[1] and 470 g Ace-hi commercial poultry concentrate.[2]

Protein-deficient diet: This was estimated to contain only 3–4% protein, but it was adequate in all other essential nutrients for the rat. It consisted of 450 g ground yellow corn, 2 ml cod liver oil, 50 ml cottonseed oil, 40 g Hegsted (23) mineral mixture,[1] 458 g cornstarch, and 50 ml of a vitamin solution which was a modification of that recommended by Manna and Hauge (24). The final concentration of vitamins in the diet was the same as described by Castellanos and Arroyave (25).

Animals.—All of the animals came from the colonies of the Institute of Nutrition of Central America and Panama. They were housed in individual cages and weighed periodically throughout the experiment.

A total of 38 Sprague-Dawley rats with a weight range of 68–98 g were used in Experiment A. These were divided into 3 groups: 7 males and 7 females in group I, 10 males and 9 females in group II, and 3 males and 2 females in group III. 1 ml of 1% croton oil was injected into experimentally produced subcutaneous air sacs in groups I and II, and 1 ml of corn oil was injected into group III. Group I received a complete diet throughout the experimental period. Groups II and III were placed on a protein-deficient diet 2 wk before the preparation of the air sac.

A total of 27 Wistar rats with a weight range of 120–214 g were used in Experiment B. These were divided into 2 groups: 7 males and 6 females in group I, and 8 males and 6 females in group II. 1 ml of 1% croton oil was injected into experimentally produced subcutaneous air sacs in both groups. Group I received the complete diet throughout the experiment. Group II was given the deficient diet 1 day after the preparation of the pouch, immediately after samples were taken for blood studies (see below). Prior to this, Group II received the same diet as group I.

Blood Studies.—Standard equipment and methods (26) were used to study the total and differential leukocyte counts of circulating blood. Blood was obtained by amputation of the tip of the tail.

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[1] Nutritional Biochemical Corp., Cleveland, Ohio.

[2] California Milling Corp., Los Angeles, Calif.

In Experiment A, samples were collected from 5 male and 5 female rats from each of groups I and II, and all of the rats from group III, 1 day before and 1, 5, and 10 days after the preparation of the pouch. For the last collection, the sample size was reduced to 5 for groups I and II and 3 for group III. In Experiment B, samples were collected from 4 male and 5 female rats from group I and 5 male and 5 female rats from group II, 1 day before and 1, 5, 8, and 11 days after the preparation of the pouch. The sample size for group II was reduced to 8, 8 days after the preparation of the pouch. In both experiments, animals for blood-testing were randomly selected.

Preparation of the Pouch.—The method used to prepare the granuloma pouch was essentially the same as described by Selye (11). Sterile technique was employed throughout the operation. The croton oil and corn oil solutions were sterilized by passage through a bacterial filter.

Transillumination.—The accumulation of exudate in the pouch lumen was observed by transilluminating the pouch in a darkened room while holding the animal in a vertical position. A microscope lamp was used as a light source.

Postmortem Examinations.—Sterile technique was used to examine all animals except those examined at 6 days in Experiment A. In Experiment A, animals were sacrificed according to the following schedule: 5 from group I, 8 from group II, and 2 from group III, 6 days after the preparation of the pouch; 5 from group I, 7 from group II, and 3 from group III, 11 days after; and the remaining 4 from each of groups I and II, 28 days after. In Experiment B, the schedule was: 4 from group I and 5 from group II, 6 days after the preparation of the pouch, and the remaining 9 from each group, 12 days after.

Exudate.—The term "exudate," as used in this study, refers to all fluid removed from the pouch at postmortem. Its volume and gross appearance were recorded, and after thorough mixing and shaking to break up large clumps, samples were taken for total and differential leukocyte counts (26). The supernatant fluid was drawn off after centrifugation at 3000 rpm for 15 min and aliquots used for determining total protein concentration by the Folin-Ciocalteau method as described by Lowry et al. (27).

Pouch.—After evacuation of the exudate, the pouch was separated from the attached tissue and weighed. It was then fixed in 10% formaldehyde for histological studies. Cross sections were taken from the middle part of the longitudinal axis of the pouch and from the middle parts of both halves. These were embedded in paraffin, and sectioned at 6 μ. In addition to staining with routine hematoxylin and eosin, additional sections were stained with Mallory's phosphotungstic acid hematoxylin (PTA) and Mallory's aniline blue (28).

The thickness of the inflammatory capsule of the pouch was measured four times with an ocular micrometer in a light microscope at 2, 4, 8, and 10 o'clock in each of three cross sections; the average of all the values obtained for each pouch was determined.

Estimates of the concentrations of polymorphonuclear (PMN) leukocytes, mononuclear cells, fibroblasts, and collagen in the pouch were made subjectively, using arbitrary values of 0 to 5+.

Bacteriological Examinations.—Except for the samples collected in the early part of Experiment A (see later), exudates or the inner scrapings of pouches that contained no exudate were cultured on blood agar for evidence of accidental bacterial infection.

Statistical Analysis.—Unless stated otherwise, the analysis of variance, employing the F test as the test of significance (29), was used to analyze the data.

RESULTS

Growth.—Text-fig. 1 presents the growth curves for groups I and II from Experiment A. The growth curves for animals from Experiment B were similar.

The curves for group III of Experiment A were practically identical with group II and are omitted from the figure. Both males and females fed the complete diet gained weight steadily. The gain was greater in males than in females. In contrast, animals fed the deficient diet gained little weight. This agrees with the findings of Castellanos and Arroyave (25). The development
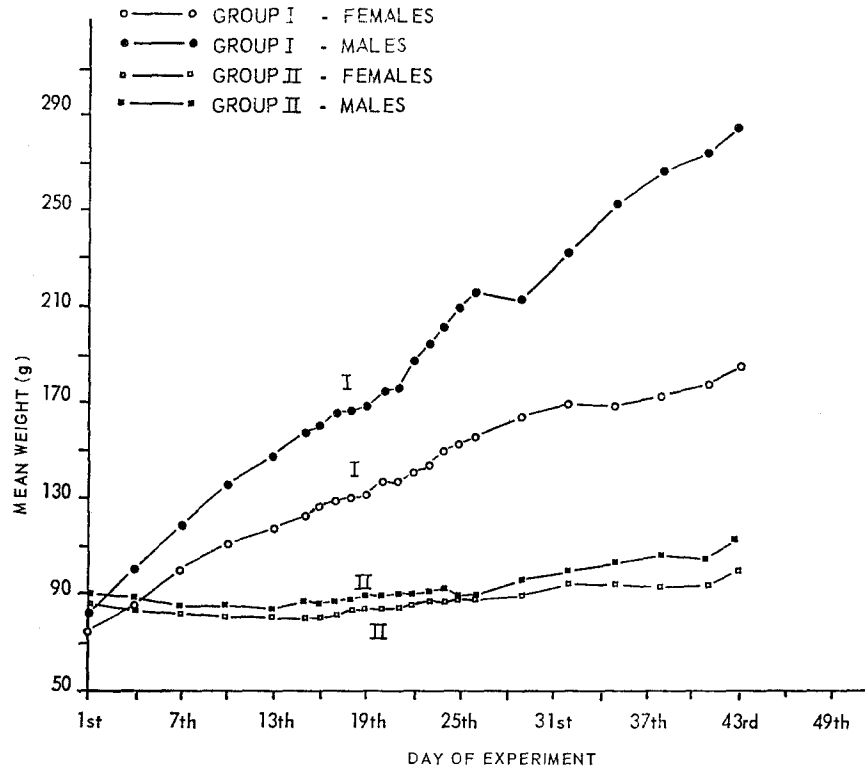

TEXT-FIG. 1. Experiment A. Growth curves of rats by groups.

of the pouch in animals given the complete diet, as described later, probably enhanced the difference between the two dietary groups.

Blood Studies.—Normal values for total and differential leukocyte counts in rats as given by Farris and Griffith (30) approximate 6000 to 18,000 leukocytes per cubic millimeter, of which 2–14% are nonsegmented and 6–25% segmented neutrophils; the remainder are essentially lymphocytes. These values are for healthy Wistar rats. It is possible that values may differ slightly for other strains.

Text-figs. 2 and 3 show the mean total and differential leukocyte counts for circulating blood of the various groups of animals.
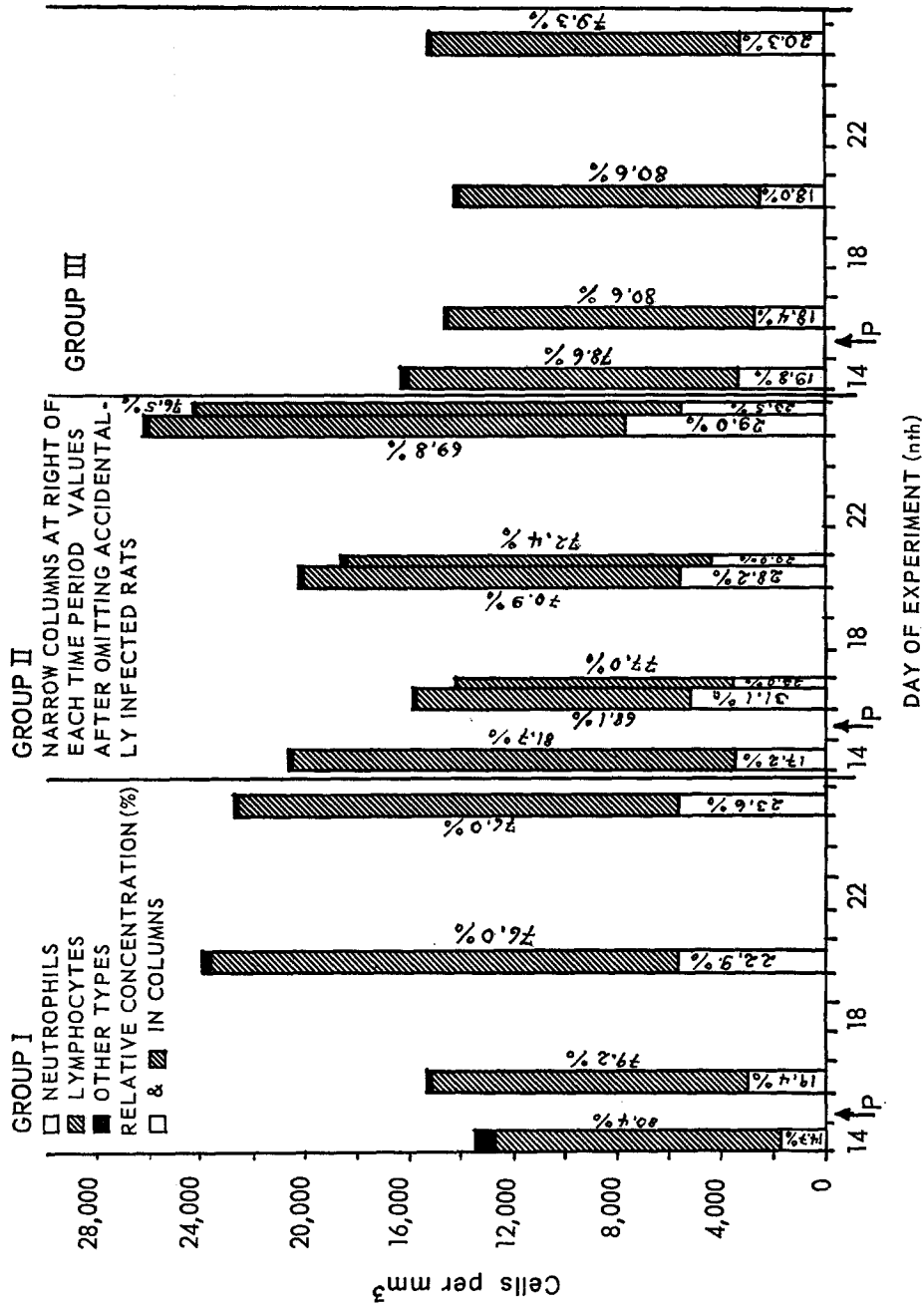
Text-Fig. 2. Experiment A. Mean total and differential counts of circulating blood.
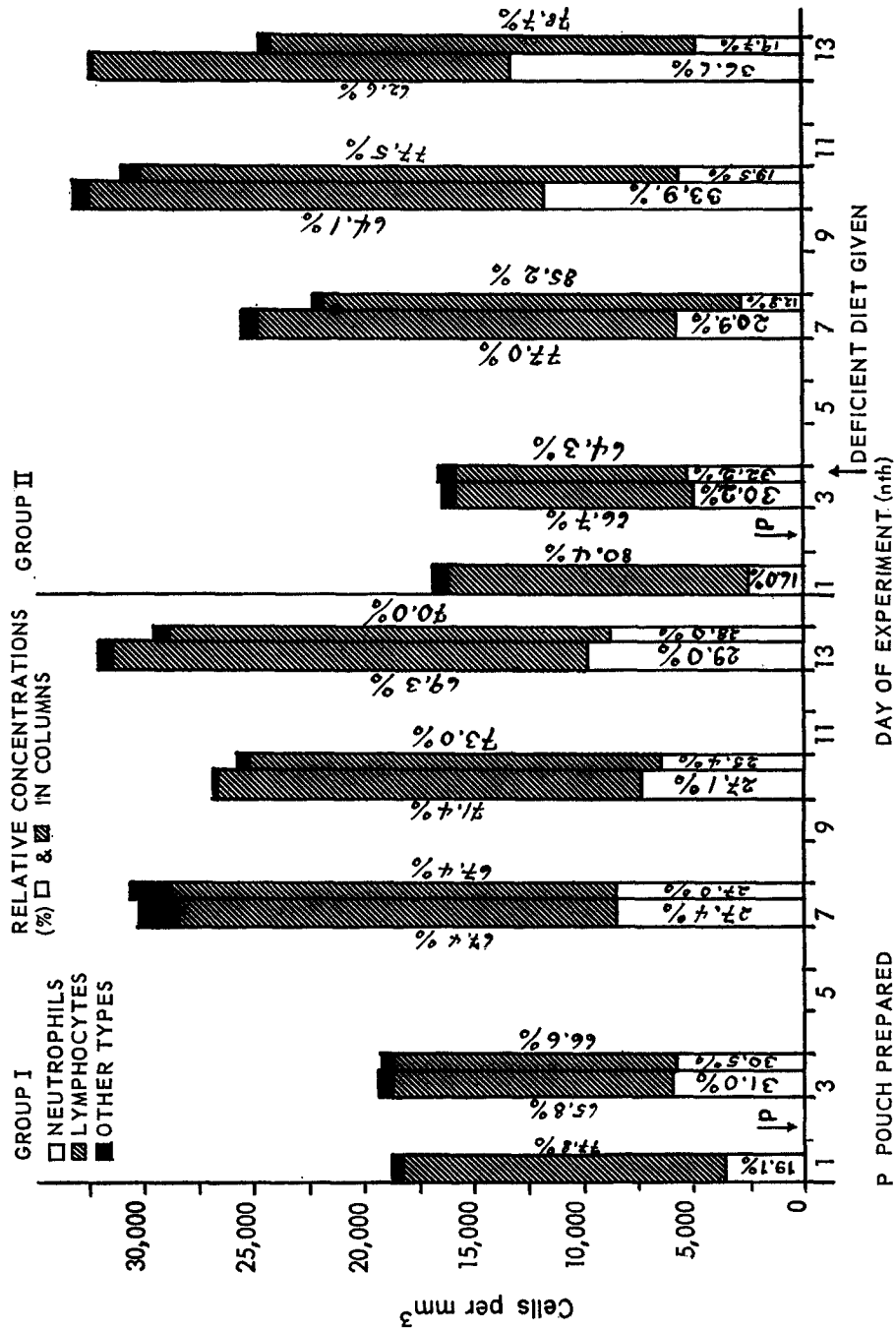
TEXT-FIG. 3. Experiment B. Mean total and differential counts of circulating blood.

Leukocytosis followed the injection of croton oil in well-nourished and mal-nourished animals of both experiments. The time trends shown were highly significant ($P < 0.01$). There was no significant difference between the over-all effects of the two dietary regimens although the initial mean count for group II in Experiment A was significantly higher than that for group I by the t test ($P < 0.01$). No satisfactory biological explanation can be offered for this. Variation in the total leukocyte counts of individual rats may be of a high order (31). Hemoconcentration due to acute protein deficiency (32) was probably not responsible because both groups had been given the same diet for the same period of time.

Group III of Experiment A exhibited no leukocytosis (Text-fig. 2) and showed only a slight difference between successive differential counts. This was probably due to the lack of irritating properties in corn oil, the inoculum used for this group (11).

Leukocytosis in both experiments was accompanied by neutrophilia and lymphocytosis, the former making a relatively greater proportional contribution than the latter (Text-figs. 2 and 3). Changes in the relative concentration of lymphocytes, for the most part, reciprocally compensated for changes in the relative concentration of neutrophils. In Experiment A, increase in the absolute concentration of lymphocytes was immediate for group I, but for group II there was first a considerable drop which accompanied the initial decrease in total leukocytes. There was no evidence of severe granulocytopenia.

Effect of accidental infection: A number of rats, mostly in group II of both experiments, showed, on postmortem examination, evidence of secondary infection in the pouch (see below). Removal of accidentally infected animals from the calculations made for group II resulted in a general lowering of the total and neutrophil counts and a slightly more marked delay in the leukocytic response (Text-figs. 2 and 3). However, because of the small number of observations left, 2–6, the statistical analysis was confined to all of the rats in group II, as discussed in the earlier section. Total and differential counts for group I of Experiment B showed only a slight alteration after removal of one accidentally infected rat (Text-fig. 3).

Characteristics of the Exudates.—

Volume: Changes in the volume of exudate in the pouches, observed by trans-illumination, paralleled those determined by objective measurements made at postmortem (Table I).

Generally, exudates were grossly visible in the pouches of well-nourished rats by the 4th day after the injection of croton oil. In some of the malnourished rats, this did not occur until 1 or 2 days later; in many, there was no evidence of exudate formation at any time during the experiment. The progressive accumulation of exudate with time proceeded more slowly in the pouches of malnourished rats than in those of the well-nourished.

No exudate was seen by transillumination in the pouches injected with corn oil, group III in Experiment A. At postmortem these yielded only 0.50 ml or less of oil.

In both experiments, the mean volumes of exudate harvested from group I were greater than those harvested from group II (Table I). The over-all difference was significant at the 1% level for Experiment A and at the 5% level for Experiment B.

Removal of accidentally infected animals from the calculations resulted in even lower mean volumes for group II in both experiments (Table I). This increased the difference between the two dietary groups.

TABLE I

Experiments A and B: Mean Total Volumes of Croton Oil-Induced Exudate

Exp.	Group	Age of pouch							
		6 days		11 days		12 days		28 days	
		n	ml ± SE	n	ml ± SE	n	ml ± SE	n	ml ± SE
A	I	5	2.54 ± 0.69	5	6.52 ± 2.40	—	—	4	2.95 ± 1.65
	II	8	0.71 ± 0.25	7	0.22 ± 0.17	—	—	4	0.16 ± 0.08
	(II)	(5)	(0.34 ± 0.17)	(4)	(0.09 ± 0.36)	—	—	—	—
B	I	4	6.12 ± 1.62	—	—	9	9.73 ± 1.96	—	—
	(I)	—	—			(8)	(9.61 ± 2.23)	—	—
	II	5	3.98 ± 0.84	—	—	9	5.33 ± 1.34	—	—
	(II)	—	—	—	—	(4)	(2.66 ± 1.8)	—	—

Group I, well-nourished rats. Group II, malnourished rats.

n, number of observations; ( ), values obtained after omitting accidentally infected rats.

Gross appearance: Generally, exudates harvested from well-nourished rats, group I, were hemorrhagic, thick, and oily. However, two from Experiment A consisted of oil, and one from Experiment B was slightly purulent.

Only 2 exudates from malnourished rats, group II of Experiment A, were hemorrhagic, 4 were purulent, and 10 consisted of oil. Three pouches contained no exudate. 8 out of 14 group II pouches from Experiment B were hemorrhagic, 3 were slightly purulent, 2 were very purulent with a thick, milky consistency, and 1 consisted of oil.

Total and differential leukocyte counts: Not all animals gave exudate samples suitable for either leukocyte counts or total protein determinations (see below). Total leukocyte counts and the relative concentration of neutrophils for purulent exudates were extremely high and made meaningful analysis difficult, especially for group II. The response in group I in both experiments was neutrophilic (Table II). The increase in neutrophil concentration was accompanied by a compensatory decrease in the relative concentration of lymphocytes. Ob-

servations made on three 28-day-old exudates from group I in Experiment A indicated that, by this time, there was a subsidence of the local response to croton oil.

Monocytes made up less than 10% of the total count and eosinophils were rarely encountered.

Total protein: In Experiment A, only groups I and II, examined 6 days after the preparation of the pouch, contained a sufficient number of observations (n)

TABLE II

Experiments A and B: Mean Total Leukocyte Count and Relative Concentration of Neutrophils and Lymphocytes in Croton Oil–Induced Exudates

Exp.	Age of pouch	Group	n	Cells per mm³ ± SE	Neutrophils	Lymphocytes
	days				%	%
A	6	I	5	12,200 ± 3,915	33.8	59.0
		II	5	39,420 ± 19,865	60.8	32.0
		(II)	(2)	(8,650 ± 5,750)	(40.0)	(57.5)
A	11	I	4	8,220 ± 2,495	60.7	39.0
		II	1	108,400	92.0	7.0
		(II)	(0)	—	—	—
A	28	I	3	4,800 ± 1,399	28.0	72.0
		II	0	—	—	—
B	6	I	4	7,270 ± 3,120	44.7	54.2
		II	5	5,040 ± 226	51.6	47.2
B	12	I	9	15,930 ± 4,260	63.4	36.6
		(I)	(8)	(15,990 ± 4,830)	(60.1)	(39.9)
		II	8	56,770 ± 15,430	75.4	24.6
		(II)	(3)	(12,630 ± 1,440)	(47.7)	(52.3)

Group I, well-nourished rats. Group II, malnourished rats.

n, number of observations; ( ), values obtained after omitting purulent exudates.

for analysis. The mean, g/100 ml ± standard error, for group I was 4.35 ± 0.14 (n = 4). It was not significantly higher than that for group II, 3.99 ± 0.62 (n = 3).

In Experiment B, the means for group I were 4.57 ± 0.25 (n = 4) and 4.70 ± 0.09 (n = 9), 6 and 12 days after preparation of the pouch. The means for for group II were 4.09 ± 0.16 (n = 5) and 4.21 ± 0.13 (n = 7), respectively. The over-all mean for group I was significantly greater than that for group II (*P* < 0.01).

Characteristics of the Pouch.—

Gross appearance: Pouches from group I of Experiment A were generally

well formed and easy to separate from the surrounding connective tissue. Pouches from group II were mostly thin walled and required more careful dissection to achieve separation (Fig. 1); they were often ruptured during the operation. In Experiment B, this difference was evident in 12-day-old pouches but not in 6-day-old specimens (Fig. 2). The difference between the two groups, in both experiments, seemed to be more marked with increasing age of the pouch, and consequently, increasing severity of the deficiency state (Fig. 3).

TABLE III

Experiments A and B: Mean Weight and Thickness of Granuloma Pouches

Exp.	Age of pouch	Group	Weight		Thickness	
			n	g ± SE	n	μ ± SE
A	*days* 6	I	5	2.514 ± 0.176	5	423 ± 61
		II	8	2.417 ± 0.339	8	251 ± 41
		(II)	(5)	(1.874 ± 0.046)	(5)	(225 ± 42)
	11	I	5	2.803 ± 0.612	5	637 ± 125
		II	7	1.429 ± 0.156	7	289 ± 56
		(II)	(4)	(1.301 ± 0.147)	(4)	(170 ± 78)
	28	I	4	1.565 ± 0.374	3*	773 ± 87
		II	4	0.199 ± 0.053	1*	754
B	6	I	4	4.609 ± 0.738	4	598 ± 84
		II	5	3.799 ± 0.394	4*	566 ± 37
	12	I	9	3.164 ± 0.398	9	899 ± 70
		(I)	(8)	(2.968 ± 0.439)	(8)	(879 ± 75)
		II	9	2.589 ± 0.461	9	779 ± 127
		(II)	(4)	(1.425 ± 0.433)	(4)	(415 ± 72)

Group I, well-nourished rats. Group II, malnourished rats.

n, number of observations; ( ), values obtained after omitting accidentally infected rats.

* Not equal to number of pouches weighed because in Experiment A some pouches nodular or ill-defined and difficult to measure; in Experiment B, pouch destroyed accidentally during fixation.

There was no definite membrane to dissect in group III of Experiment A. In the gross, the tissue with which the corn oil had contact appeared normal.

Weight: The over-all mean weight of pouches from group I was significantly greater than those from group II (Table III) in Experiment A ($P = 0.05$), but not in Experiment B. There was a significant decrease in weight with age of the pouch in both experiments ($P = 0.05$), but the trends in each dietary group in Experiment A were different ($P < 0.01$).

When rats suspected of accidental infection (see below) were removed from the calculations, there was a decrease in the mean for each group involved and an increase in the difference between the two dietary groups (Table III).

Histological appearance: Microscopically, pouches consisted of an inflammatory capsule, the reactive section where inflammation and active collagen formation were occurring, and surrounding fatty tissue which could not be separated from the capsule on dissection. The histological description applies to the capsule. Results correlated with the macroscopic appearance of the pouches.

All of the 6-day-old pouches from group I in Experiment A and from both groups in Experiment B possessed capsules that were histologically well defined around the whole circumference of the pouch lumen (Fig. 4a–b). The connective tissue was compact, and there were some inflammatory cells that were mainly mononuclear; PMN leukocytes were seen in small numbers in two of the pouches from Experiment A (Fig. 4c–e). Generally, group II pouches from Experiment A had poorly-formed capsules at 6 days, consisting entirely or partially of loosely formed connective tissue with many pseudocysts that seemed to contain oil (Fig. 4a–b). Both mononuclear cells and PMN leukocytes were evident, the former being made up essentially of macrophages, many of which showed a foam cytoplasm due to phagocytosed oil; there was a relative abundance of PMN leukocytes in 4 of 8, 3 of which were suspected of being accidentally infected (Fig. 4c–e).

Young, active fibroblasts were prominent in most of the 6-day-old pouches from both experiments. However, fibroblastic activity and, consequently, collagen production was greater in 11- and 12-day-old pouches. Well-nourished group I animals in this older age group generally had well-formed capsules consisting of young, active fibroblasts; there were very few mononuclear cells and practically no PMN leukocytes (Fig. 6). However, three from Experiment B had doubly encapsulated walls similar to that shown in Fig. 5, and one was reduced to a scar nodule in which the inflammatory reaction had almost completely subsided. 11-and 12-day-old pouches from malnourished group II animals, on the other hand, had, for the most part, loosely organized capsules with fewer fibroblasts, less collagen, and more mononuclear cells than group I (Fig. 6). Three from Experiment A and five from Experiment B showed marked infiltration by PMN leukocytes and were suspected of being accidentally infected.

One of the 28-day-old pouches from group I in Experiment A was reduced to a scar nodule. The remainder were well defined with doubly encapsulated walls (Fig. 5) made up almost entirely of mature fibroblasts and collagen fibres. In group II, two pouches were nodular but still contained some inflammatory (mononuclear) cells. Collagen formation was moderate in both but fibroblastic activity was prominent in only one. The remainder were made up essentially of fibroblasts and collagen; one was well encapsulated, the other incompletely encapsulated.

Thickness of inflammatory capsule: Results obtained in Experiment A, 28 days after pouch formation, were not used in the statistical analysis. The

over-all mean thickness (μ) of group I capsules was significantly greater ($P < 0.01$) than that of group II capsules in Experiment A, but not in Experiment B (Table III). However, when accidentally infected rats were removed from the calculations, the difference was enhanced and became highly significant ($P < 0.01$) in Experiment B.

Accidental Infections.—1 out of a total of 27 well-nourished rats, group I in both experiments, and 11 of 33 malnourished rats, group II, showed evidence of secondary infection of the pouch. This difference was significant by the chi-square test ($P = 0.05$). Such infections were first suspected when three 6-day-old exudates from group II of Experiment A were found to be purulent. They were not cultured bacteriologically, but Gram-positive cocci, resembling pneumococci in one instance and staphylococci in the other, were seen in smears made from two of the exudates. The smear made from the third exudate was negative. However, it was not as thickly purulent as the other two, and actually appeared slightly pink and watery. Sterile technique was not used in these initial dissections, but it was employed in all subsequent postmortem examinations, and all exudates or scrapings of pouches containing no exudate were cultured on blood agar. If cultures were negative, the macroscopic and microscopic characteristics of the exudates, the histological appearance of the pouch, and the hematological findings were used to give supportive evidence for secondary infection.

The remaining eight positives from group II were examined 11 and 12 days after preparation of the pouch. Three were from Experiment A and yielded strains of nonhemolytic staphylococci, Gram-negative diplocci, and hemolytic staphylococci, respectively. Five were from Experiment B; *Staphylococcus aureus* was isolated from one and nonhemolytic streptococci from another. Primary cultures from the remainder showed a few (2–5) very small colonies after 48–72 hr incubation at 37°C. They were lost on subculture probably because the cultural conditions were unsuitable. A satisfactory smear could be prepared from only one of the original plates. It contained diphtheroid-like organisms.

The one positive rat from group I behaved in a similar manner to the above, and no bacterial strain was isolated. This animal was examined 12 days after preparation of the pouch and came from Experiment B.

DISCUSSION

In contrast to the severe and prolonged protein deficiency necessary to bring about an impairment of mechanisms of defense such as antibody production (33), relatively short periods of dietary inadequacy were found in the present experiments to inhibit the local inflammatory response in rats. There was also a delay in tissue repair similar to the effect of protein depletion on the healing of open wounds (18–20, 34, 35). The degree of development of the

granuloma pouch (11) was used as a measure of inflammation. The mechanism by which protein affects this development is uncertain. The similarity between the histological and gross appearance of the pouch in malnourished animals and that described by Selye (11) in adequately nourished rats whose pouch walls were injected with hydrocortisone is suggestive, and points to hormonal imbalance as one possibility. Endocrine disturbances have been reported in malnourished humans (36–39), and it is possible that the deficiency state used in this study caused an imbalance in the pituitary and adrenal hormones shown both to regulate inflammation (11, 40) and to play an important role in infectious processes (41). Furthermore, protein may be required locally by the proliferative elements in the area of inflammation. It may also be needed "at a distance" from the site of the inflammatory reaction, as a source of nitrogen either to replace the nonspecific nitrogen loss that occurs as a result of injury (42), or to maintain other metabolic processes involved in the inflammatory response and tissue repair.

Laboratory rats are usually fairly resistant to postoperative infections (30, 43). However, in spite of the aseptic precautions taken during preparation, the pouches of more than 30% of the malnourished rats gave evidence of accidental infection, in contrast to only 4% of the well-nourished rats. It seems that in well-nourished animals, which suffered no apparent inhibition of the inflammatory and local tissue response, the local reaction either suppressed the small number of infective organisms inadvertently introduced while preparing the pouch, or prevented the invasion of the pouch by microorganisms previously located in other parts of the body. These results are similar to those reported by Allison and Fitzpatrick (44) and parallel observations made in humans with low serum protein levels (45). They present further evidence in support of a synergistic relationship between malnutrition and infection.

Although there was a suggestive delay in the leukocytic response of malnourished animals following the injection of 1% croton oil, the height of the response was equal to or greater than that shown by animals fed the complete diet, and neutrophilia accompanied leukocytosis. These results differ somewhat from those obtained by other investigators (13–16, 46). Malnourished animals, under the present conditions, were also able to respond to accidental infection by a more marked leukocytic response, and an enhanced local response. Infection was generally associated with a relative abundance of PMN leukocytes in the pouch wall, a purulent exudate, and in some instances an increase in the volume of exudate recovered and/or the thickness of the capsule and weight of the pouch. The part played by increased stress (47) and hemoconcentration (32) in the production of high counts by malnourished animals in the latter part of the experiments is unknown. It is possible that the induced malnutrition acted as a slow-acting stress (48).

The results of the present experiments illustrate the value of the granuloma

pouch as an experimental procedure for studying the basic mechanisms under-lying the interrelationship between nutrition and infection, particularly the dietary factors regulating inflammation and wound healing. Previous studies, involving the production of experimental wounds in laboratory animals, have been directed at the latter part of the inflammatory process, i.e., the repair stage which is characterized by the proliferation and maturation of fibroblasts. Although this has yielded valuable information on the course of wound healing under various conditions, it has certain limitations (49). Selye's granuloma pouch technique (11) allows the study of both the initial, acute local tissue reaction, and the subsequent repair stage. Quantitative data may be obtained in terms of the weight of the granuloma tissue, the thickness of the inflam-matory capsule, and the volume of the inflammatory exudate; and one can distinguish accurately between newly formed and adjacent "old" tissue. Even in the case of malnourished animals, whose pouches were often poorly formed, separation of the reactive tissue was possible. Samples for the examination of the gross and microscopic characteristics of the accumulated exudate and the newly formed granuloma tissue, obtained from rats sacrificed at various times during the experimental periods, exhibited considerable uniformity. Furthermore, the isolation of bacteria from the pouch, in the case of accidental infection, suggests that it may well serve as a type of in vivo tissue culture system reminiscent of the various cavities of the chick embryo for the culti-vation of viruses and other microorganisms; and as such, could be used to study the effect of malnutrition on various local reactions during infection: growth of experimentally inoculated microorganisms, specific and nonspecific antibody response, histochemical and metabolic reactions.

SUMMARY

In the present experiments, Selye's granuloma pouch technique was applied to the study of the effect of host nutritional state on inflammation and the local tissue response. The normal response of well-nourished laboratory rats fed a diet containing 28% protein to the injection of 1% croton oil into a preformed subcutaneous air sac involved the accumulation of hemorrhagic exudate in the pouch lumen and the progressive thickening of the pouch wall, with the proliferation and maturation of fibroblasts and the eventual laying-down of collagen. In malnourished animals, fed a diet containing only 3-4% protein but adequate in all other nutrients, the above reactions were inhibited. This inhibitory effect was encountered after a relatively short period of de-ficiency and became more marked as the deficiency progressed. No consistent, clear-cut difference was seen in the leukocytic or neutrophilic response between the two dietary groups after the injection of 1% croton oil.

A significantly higher proportion of accidental bacterial infections was found in the pouches of malnourished animals than in those of well-nourished

animals. This was considered to be a possible consequence of the depressed inflammatory response in malnourished rats.

The advantages of the granuloma pouch as an experimental procedure for the study of local reactions to different noxae, and the influence of malnutrition on these reactions have been discussed and suggestions for future studies presented.

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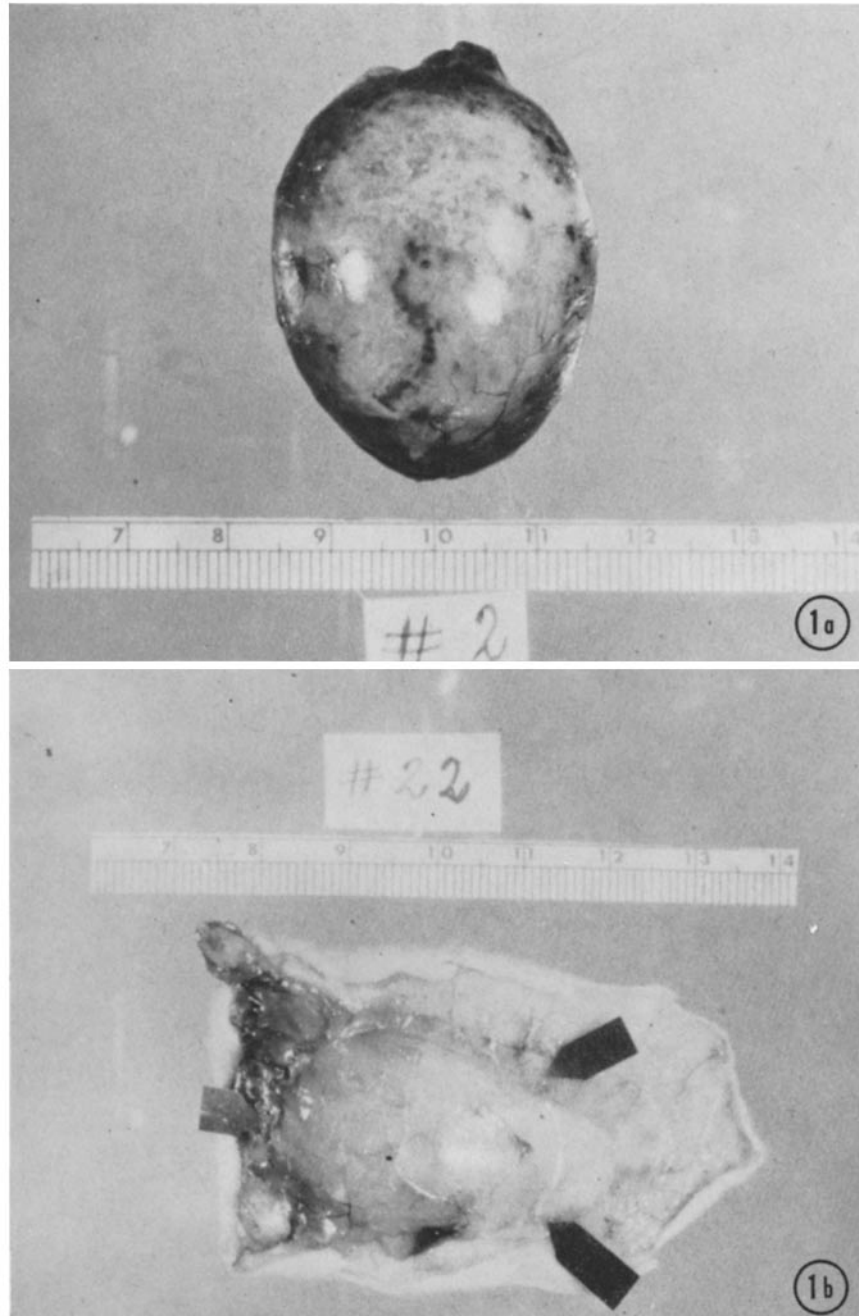
EXPLANATION OF PLATES

PLATE 41

FIG. 1. Experiment A. 11-day-old pouches from groups I and II.
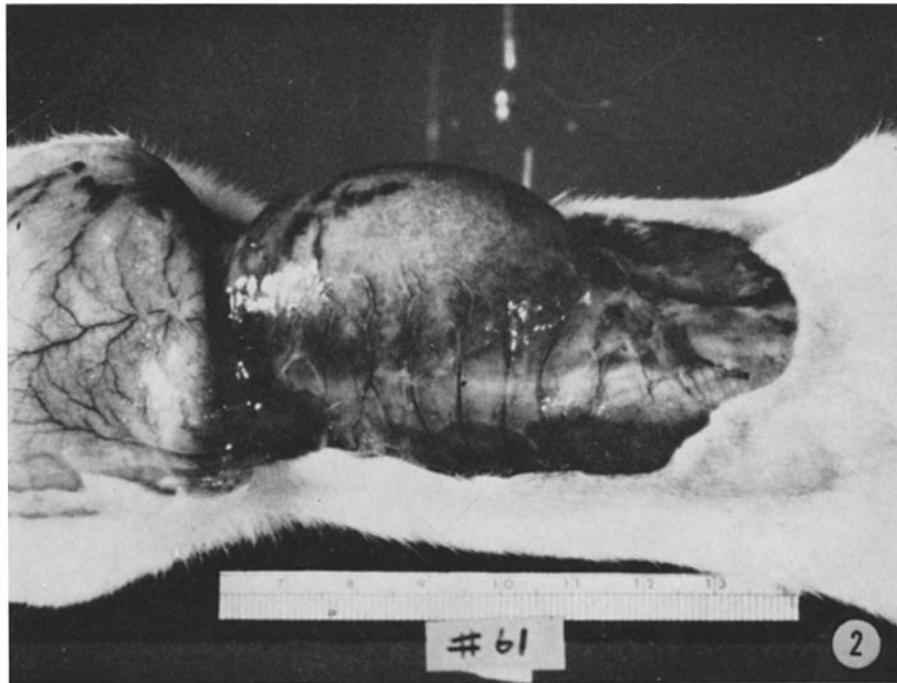
FIG. 1 a Well-nourished (group I) rat.

FIG. 1 b. Malnourished (group II) rat, shown attached to dorsal skin.

(Taylor et al.: Malnutrition and the inflammatory response)

FIG. 2. Experiment B. 6-day-old pouch in situ with dorsal skin removed. Rat given deficient diet one day after preparation of pouch (group II); well-nourished rats (group I) had pouches similar in appearance. Note well-developed vascular system serving pouch.

(Taylor et al.: Malnutrition and the inflammatory response)

FIGS. 3 a–b. Experiment A

FIG. 3 a. 28-day-old pouch of well-nourished (group I) rat, in situ after removal of covering dorsal skin. Cords of connective tissue seen emerging from the dorsal aspect of the thorax and ascending vertically towards pouch.

FIG. 3 b. Dissected 28-day-old pouch of malnourished (group II) rat still attached to dorsal skin.
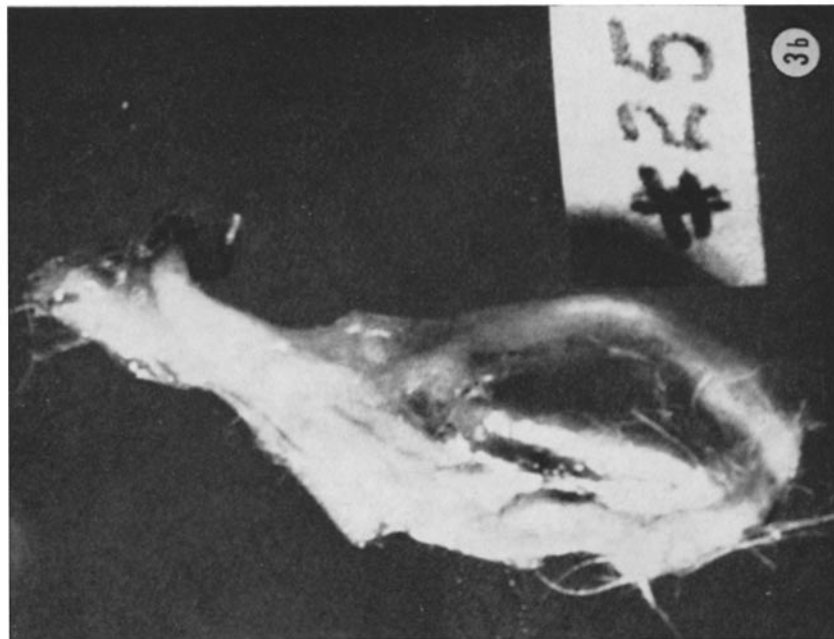
(Taylor et al.: Malnutrition and the inflammatory response)

FIGS. 4 *a–b*. Experiment A. Histological appearance (hematoxylin and eosin, obj. 3.5, oc. 10) of 6-day-old pouch walls of a well-nourished (group I) rat (Fig. 4*a*) and a malnourished (group II) rat (Fig. 4*b*).

FIGS. 4 *c–e*. Experiment A. Histological appearance (hematoxylin and eosin, obj. 25, oc. 10) of 6-day-old pouch wall of a group I rat (Fig. 4 *c*) and malnourished rats (Figs. 4 *d–e*). Relative abundance of collagen fibres in Fig. 4 *c*, virtual absence in Fig. 4 *d*. Marked PMN leukocyte infiltration in Fig. 4 *e* which yielded a purulent exudate containing organisms resembling pneumococci.
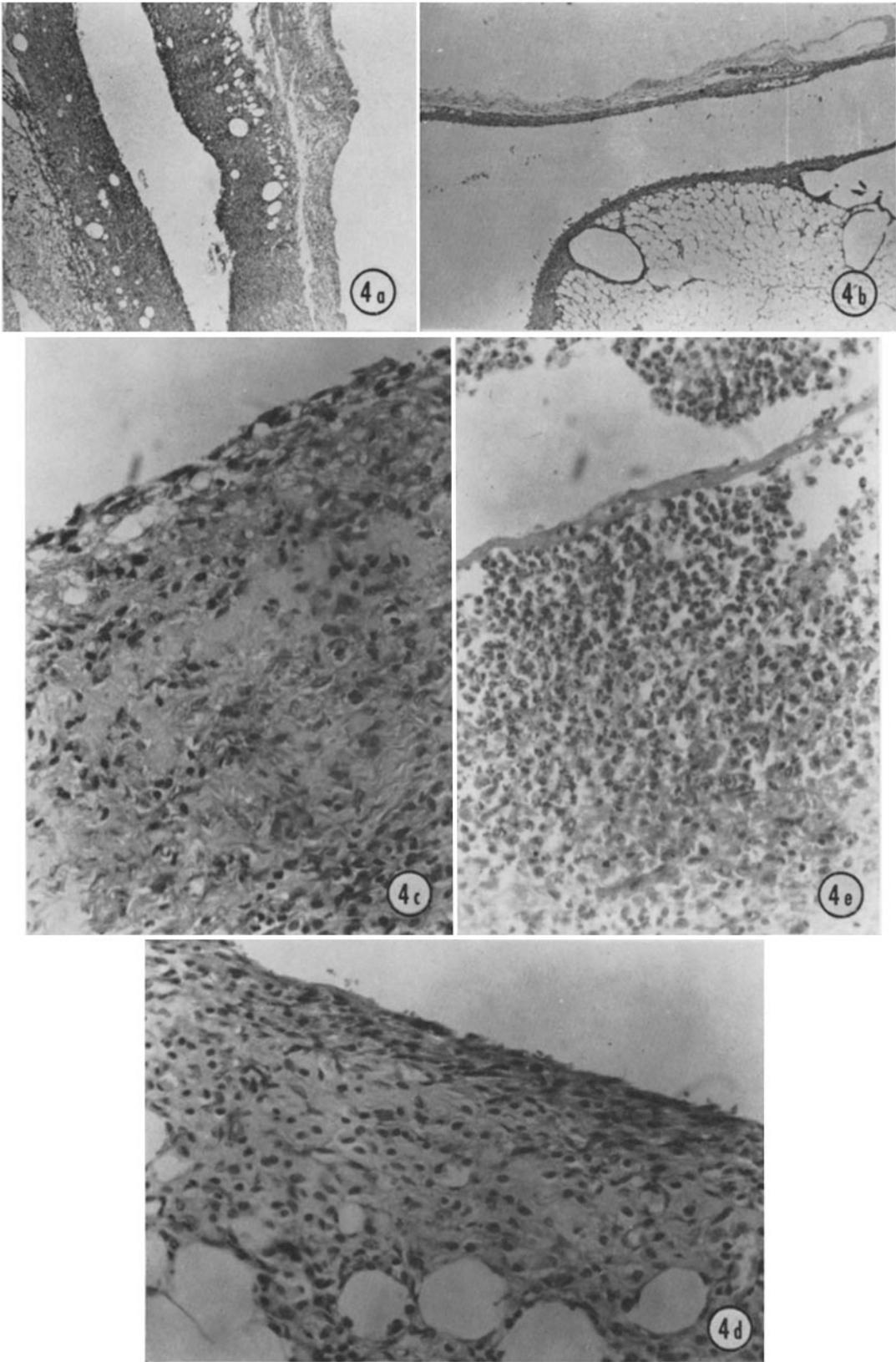
(Taylor et al.: Malnutrition and the inflammatory response)

FIGS. 5 *a–b*. Experiment A

FIG. 5 *a*. Low power (hematoxylin and eosin, obj. 3.5, oc. 10) appearance of doubly encapsulated wall of a 28-day-old pouch from group I.

FIG. 5 *b*. High power (hematoxylin and eosin, obj. 10, oc. 10) appearance of same wall.
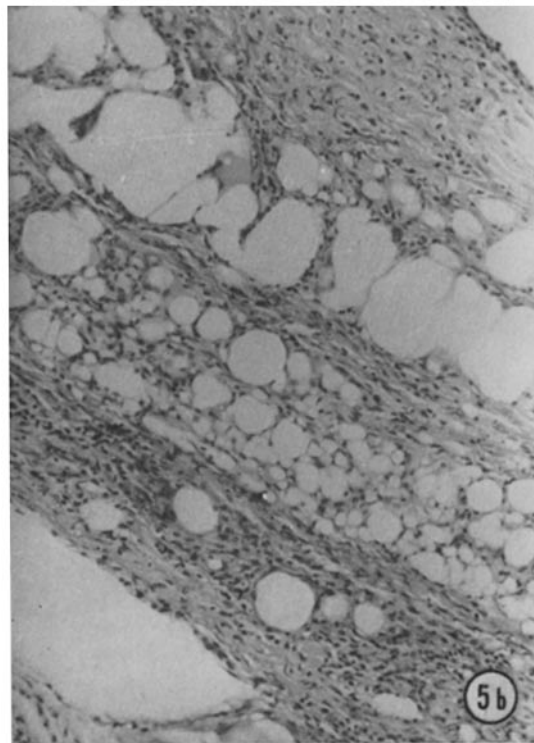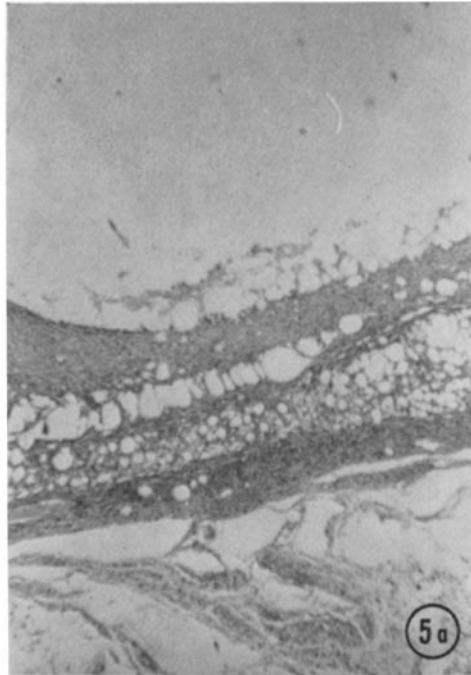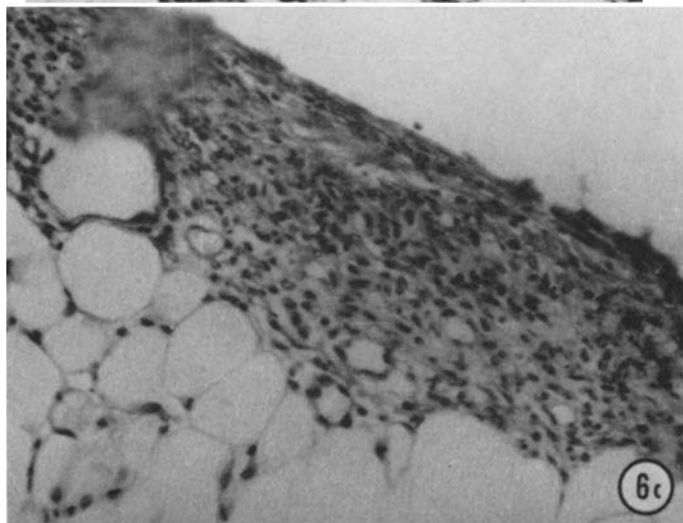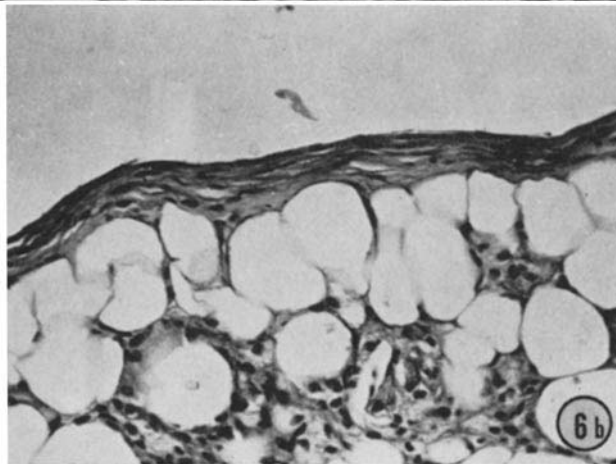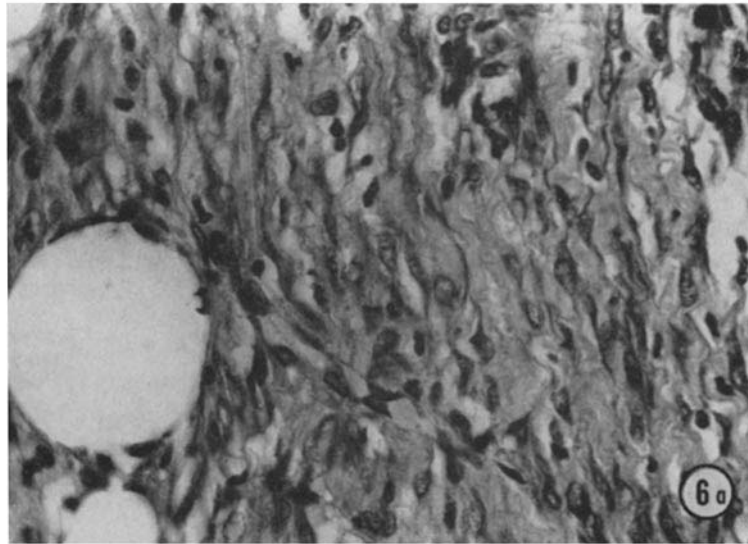
(Taylor et al.: Malnutrition and the inflammatory response)

FIG. 6 *a–c*. Experiment A. Histological appearance of 11-day-old pouch walls of group I rat (Fig. 6 *a*) (hematoxylin and eosin, obj. 45, oc. 10) and group II rats (Figs. 6 *b–c*) (hematoxylin and eosin, obj. 25, oc. 10).

Note marked fibroblastic activity and abundance of collagen fibres in Fig. 6 *a* in contrast to Figs. 6 *b* and *c*.

(Taylor et al.: Malnutrition and the inflammatory response)