The effect of malnutrition on the inflammatory response

As Exhibited by the Development of the Granuloma Pouch of the Rat after the Inoculation of Bacteria[1]

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SUMMARY

The present experiments describe the effect of host nutritional state on inflammation and the local tissue response of the rat after the inoculation of S. aureus into the granuloma pouch. Higher concentrations of the test organism were encountered in the pouch exudates of animals fed a deficient diet containing 3 to 4% protein, than in those given a complete ration, containing approximately 28% protein. Also, bacterial invasion of the pouch wall was observed in 3 malnourished rats but in none of the well-nourished. These observations were considered to be possible consequences of the depressed inflammatory response found in malnourished rats.

Pyogenic membrane formation was found in a significantly higher proportion of malnourished infected rats than from well-nourished animals. This may have been a reflection of fundamental differences in the physiological processes required by malnourished rats to handle infection in the

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pouch. Furthermore, a significantly higher proportion of malnourished rats were found to be accidentally infected with a wide variety of micro-organisms. However, no significant difference was found in the systemic response of the 2 dietary groups to the various type of inoculum used.

INTRODUCTION

Earlier studies in rats (1) have shown that malnutrition, induced by feeding a low-protein diet, is accompanied by an inhibition of local inflammation, as exhibited by the development of the granuloma pouch of the rat (2). This inhibition, it was felt, resulted in an increased susceptibility to accidental infection in the pouch. The present paper describes the local and systemic reactions in experimental rats following the inoculation of bacteria into the granuloma pouch.

MATERIALS AND METHODS

Experimental Groups. The source, handling and care of the experimental animals, the composition of the diets and the preparation of the granuloma pouch by the subcutaneous injection of croton oil have been described (1). Male, Sprague-Dawley rats, 4 to 5 weeks old, with a weight range of 90-184 g were subdivided into 4 groups. Groups I and II received a complete diet, containing approximately 28% protein, throughout the experiment. Groups III and IV were changed from the complete diet to a deficient diet containing 3 to 4% protein, 3 days before the preparation of the pouch. Groups I and III were inoculated with bacteria while groups II and IV received a non-viable inoculum. The period of dietary deficiency was selected in order to produce a moderate deficiency state (3) in the malnourished rats by the time the adaptive mechanisms were called upon to handle the experimental infection produced in the pouch 5 or 6 days later.

In earlier studies (1), the experiments were referred to as A and B. The experiments described in the present paper will be referred to as C and D. In experiment C, there were 11 and 13 animals in groups I and III respectively and 8 animals in each of groups II and IV. Animals were sacrificed and examined according to the following schedule: 4 from groups I and III and 2 from groups II and IV, 1 day after inoculation; 4 from groups I and III and 3 from groups II and IV, 3 days after; and the remainder 6 days after. In experiment D, there were 13 animals in each of groups I and III and 7 in groups II and IV. Two animals from each of groups I and III were sacrificed 1, 2, 3, 4, 5 and 6 days after inoculation, and the remaining one 13 days after. One animal from each of groups II and IV was sacrificed at the above times. Preliminary examination indicated that the statistical analysis gave more meaningful results when the data were grouped into 4 periods - 1 to 2, 3 to 4, 5 to 6 and 13 days after the introduction of the test inoculum.

Replacement of Croton Oil Exudate with Test Inoculum. The test organism was a strain of *Staphylococcus aureus* isolated on blood agar from the purulent exudate of a malnourished rat from experiments described earlier (1). Immediately after isolation, a stock culture was prepared on Difco[5] stock culture medium. Bacterial suspensions for inoculation into the pouches of groups I and III were made from nutrient agar slants seeded directly from the stock culture. After 18 hours growth at 37°C; the bacteria were suspended in isotonic saline solution, washed once and resuspended in 10 ml of fresh diluent. Thorough shaking served to break up any large clumps. Viable bacterial counts were made by means of agar pour plates (4). One ml of original suspension + 4.0 ml 6% dextran (Intradex[6]) were inoculated into the pouches of groups I and III. In experiment C, approximately 120×10^7 bacteria were inoculated into each pouch, while in experiment D, each pouch received approximately 197×10^7 bacteria. Groups II and IV received 1.0 ml sterile saline + 4.0 ml 6% dextran.

In preliminary studies using isotonic saline solution alone as diluent, there was a rapid disappearance of the fluid phase of the inoculum from the pouches of malnourished rats. This made comparative, quantitative studies between the 2 nutritional groups difficult. Six % dextran, which has been found useful as a plasma expander (5, 6) was incorporated into the diluent in a attempt to maintain an osmotic gradient between

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the extravascular and the pouch fluids, sufficient to promote retention of fluid in the pouch.

Five days after the preparation of the pouch in experiment C and 6 days after in experiment D, the croton oil-induced exudate was removed, and the inner surface of the pouch washed once with 10.0 ml isotonic saline. After removal of the saline, 10.0 ml air was introduced in order to re-form the pouch, which was then injected with the test inoculum. The site of insertion of the needle was sealed with a drop of collodion. The preparation and inoculation of bacteria were carried out after the injection of pouches receiving the non-viable inoculum, and the whole procedure took place on the same day.

Exudate formed as a result of croton oil injection is labeled exudate "1". The fluid accumulated in the pouch subsequent to the introduction of the test inoculum is referred to as exudate "2".

Blood Studies. Tail blood samples were obtained from all groups of animals at various times during the experiments, for determining total and differential counts. The sample size for each group was progressively decreased as animals were sacrificed.

Exudate "2". Exudate "2" was harvested in essentially the same way as exudate "1" (1). After thorough mixing and shaking to break up large clumps, samples were taken for one or more of the following determinations: cytological characteristics, phagocytic activity and bacterial content. After centrifugation at 3,000 r.p.m. for 15 mins., the supernatant was removed and stored at —20°C; selected samples were later examined by starch gel electrophoresis; these results will be presented in a separate publication.

Exudate "2" or the inner scraping of pouches that contained no exudate "2" were cultured on blood agar for evidence of accidental bacterial infection. In addition, exudates "2" from experimentally inoculated pouches were cultured on Chapman Stone medium[7] for the reisolation of the test organism. Viable bacterial counts were determined by the same method described for the original suspension. Serial dilutions of the exudate "2" containing bacteria were made in sterile 0.85% saline solution. It is realized that this may not have

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given a true indication but actually an underestimate of the concentration of bacteria because of large numbers of intracellular organisms. Rupture of some of the cells by vigorous shaking of exudate "2" before taking the test aliquot, and the possible loss of viability of intracellularly-located organisms helped to reduce the error.

Coverslips for the determination of the cytological characteristics of exudate "2" and *in vivo* phagocytic activity were prepared and stained in the same way as for differential counts for circulating blood. The concentration of erythrocytes, polymorphonuclear neutrophils (p.m.n) and mononuclear cells relative to each other were estimated subjectively for each smear under high power in a light microscope, using an arbitrary grading system of 0 to 10 units. Five fields were read if the relative concentrations in each field were fairly uniform. However, if they were not, more fields were read. The presence of macrophages, if noticeable, was recorded. The cellularity, i. e., the concentration of nucleated cells in each smear, relative to the remaining smears, was also estimated subjectively using a grading of 0 to 5+, by quickly scanning the preparation under low power. Similary, the concentration of bacteria was noted, and their intra-and extracellular concentrations estimated under high power. These examinations were carried out in order to give some indication of the ability of the animal to mobilize the leucocytes needed for local defense at the site of the inflammatory lesion; and to determine if diet influences the cellular content of the inflammatory exudate.

The abundance of bacteria and nucleated cells in exudate "2" smears, estimated by the method described is not strictly comparable from smear to smear because exactly equivalent volumes of exudate "2" were not used in all preparations. However, it was hoped that the results obtained would support suggested tendencies derived from other observations and to indicate additional trends.

Pilot studies showed that neutrophils were the predominant phagocytic cell type in purulent exudates obtained from the pouch. Active macrophages were seen in comparatively small numbers. Consequently, phagocytic activity was measured under high power by determining the percentage of neutrophils containing bacteria. This activity is qualified by the concentration of bacteria remaining extracellular, because it depends not only on the competence of the phagocytic cells but also on their relative abundance with respect to the concentration of bacteria present. Many studies on the phagocytic activity of neutrophils have employed *in vitro* test systems such as those utilized by Cottingham and Mills (7) and Berry *et al.* (8). In the present studies, the phagocytic cells carry out their activities within the animal body, in the medium of the pouch. This eliminates many of the variables involved in estimating the activity of the cells after removal from their natural environment and after subjecting them to laboratory treatment. However, it is realized that the method takes into consideration only the intracellular location of bacteria and not their subsequent destruction.

Histological Examinations. In addition to the routine histological examination of the pouch (1), the type of exudate present in the pouch section was noted. Exudates containing relatively large amounts of fibrin are termed fibrinous; those containing, in addition, high concentrations of neutrophils are termed fibrinopurulent. Specimens of kidney and in some cases liver and spleen were taken in order to study the histopathological effects, if any, of dextran.

Statistical Analysis. Unless otherwise stated, the data were analyzed by the analysis of variance using the F test as the test of significance (9).

RESULTS

Blood Studies. Leucocytosis following the injection of croton oil was evident in all groups of both experiments except group IV of experiment C (Figures 1 and 2). This was generally accompanied by somewhat transient increases in the relative and absolute concentrations of neutrophils.

Further increases in both total and neutrophil counts followed the introduction of the test inoculum into the pouch. The overall time trend for the total counts in each experiment was highly significant ($P<0.01$), but no statistically significant difference was found between the individual group trends nor between the effects of the 2 inocula on the overall mean count. The dietary effect was significantly different only in experiment C ($P<0.01$). This probably stemmed from a

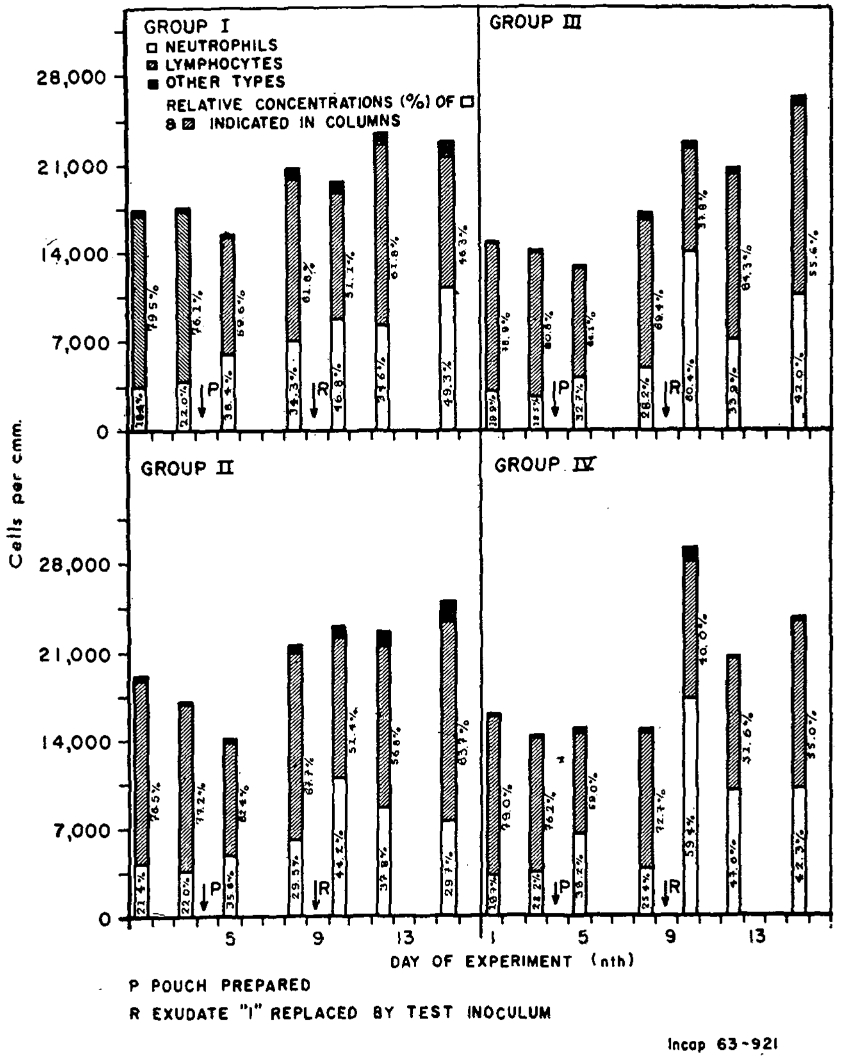

Fig. 1: Experiment C. Mean total and differential counts of circulating blood.

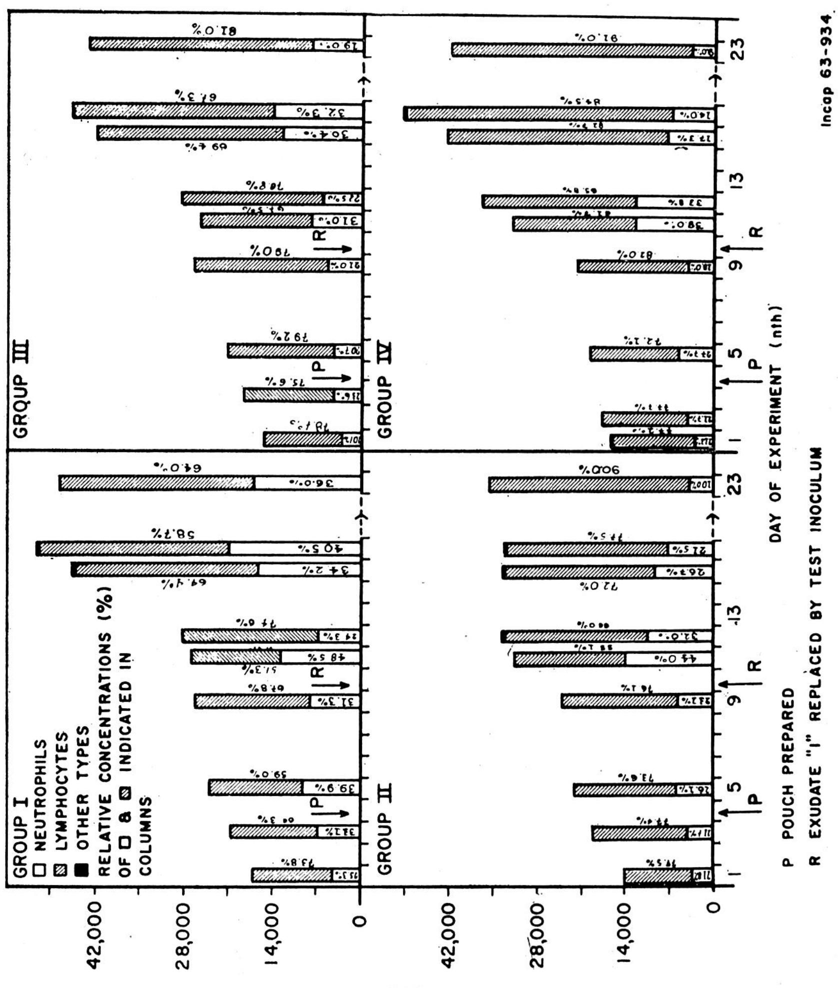

Fig. 2: Experiment D. Mean total and differential counts of circulating blood.

chance initial difference of more than 2,000 cells per cm between the overall mean for the well-nourished groups I + II, 18,080 ± 1,010, an dthe malnourished groups III + IV, 15,350 ± 740. This difference was apparent until the 8th day of the experiment, and was sufficiently great not to be cancelled out by the high maximum counts attained subsequently by the malnourished groups. In both experiments, the injection of saline-dextran into groups II and IV appeared to produce a more immediate response than the inoculation of bacteria-saline-dextran into groups I and III. In the latter groups, there may have been an immediate demand for circulating cells at the site of inoculation with a consequent masking of initial increases in the circulation.

Reciprocal compensation for the variation in relative concentration of neutrophils stemmed essentially from variation in the relative concentration of lymphocytes. In certain groups an initial decrease in the absolute concentration of lymphocytes after the injection of croton oil and/or the introduction of the test inoculum into the pouch was followed by an increase to values equal to or greater than those obtained before the preparation of the pouch (Figures 1 and 2). The decrease noted may have been a reaction to increased stress caused by the introduction of the test inoculum, and is in accord with the "General Adaptation Syndrome" of Selye (10).

The effects of the 2 inocula on the differential counts in experiment D appeared to be dissimilar. Saline-dextran resulted in a progressive decrease in the absolute concentration of neutrophils in groups II and IV from high post-injection values, and a continuous increase in the absolute concentration of lymphocytes. Bacteria-saline-dextran resulted in an intermittent increase in neutrophils in groups I and III, and successive increases in lymphocytes after the post-inoculation decreases mentioned above. In experiment C, there was a similar but less well-defined difference.

Re-calculation of the mean total and differential counts after omitting accidentally infected rats (see below) did not result in any notable change in the trends observed.

Characteristics of the Exudate.

Volume: The accumulation of exudate "1" in the pouch generally followed the pattern already described (1). In experiment C, the formation of exudate "1" was less marked than in experiment D. Although the batch of 1% croton oil used in experiment C for the preparation of the pouch was able to stimulate a system leucocytic-neutrophilic response (Figure 1), its potency in promoting local exudates formation appeared to be reduced. This may have occurred during storage of the concentrated croton oil or during its dilution with corn oil and subsequent sterilization.

Figure 3 gives the mean volumes of exudate "2" harvested at post-mortem from animals in experiment D. The pattern obtained for experiment C was similar. Observations made at 13 days after the introduction of the test inoculum are not included in Figure 3, and were not used in the statistical analysis; only 1 of the pouches examined at this time (from group II) contained a measurable volume of fluid (5.0 ml).

Irrespective of the type of inoculum the groups that had received the complete diet yielded more exudate "2" than the corresponding groups fed the deficient diet. The overall difference was significant ($P=0.05$ in experiment C and $P=0.01$ in experiment D). The means for groups III and IV decreased progressively with time, whereas those for group I remained above the volume of inoculum introduced and group II increased with time. Furthermore, irrespective of the type of diet, the overall mean in both experiments for groups inoculated with bacteria was significantly greater ($P<0.01$) than for groups injected with saline-dextran.

The removal of values for accidentally infected rats in groups II and IV resulted in a decrease in the means by 1.0 to. 6.0 ml. This parallels the observations made in previous studies (1) that accidental infection in the pouch is associated with an increased accumulation of exudate "1".

Gross appearance: Because of active bacterial proliferation in the pouches as a result of experimental inoculation, all of the exudates "2" from groups I and III were purulent. Those from group III tended to be more viscous and opaque than those from group I. The one pouch from each of groups I and III from experiment D, examined 13 days after inoculation, yielded rather dry-looking lumps of pus which could not be broken up easily by shaking in isotonic saline solution. A number of the early exudates "2" had a slightly pinkish tinge

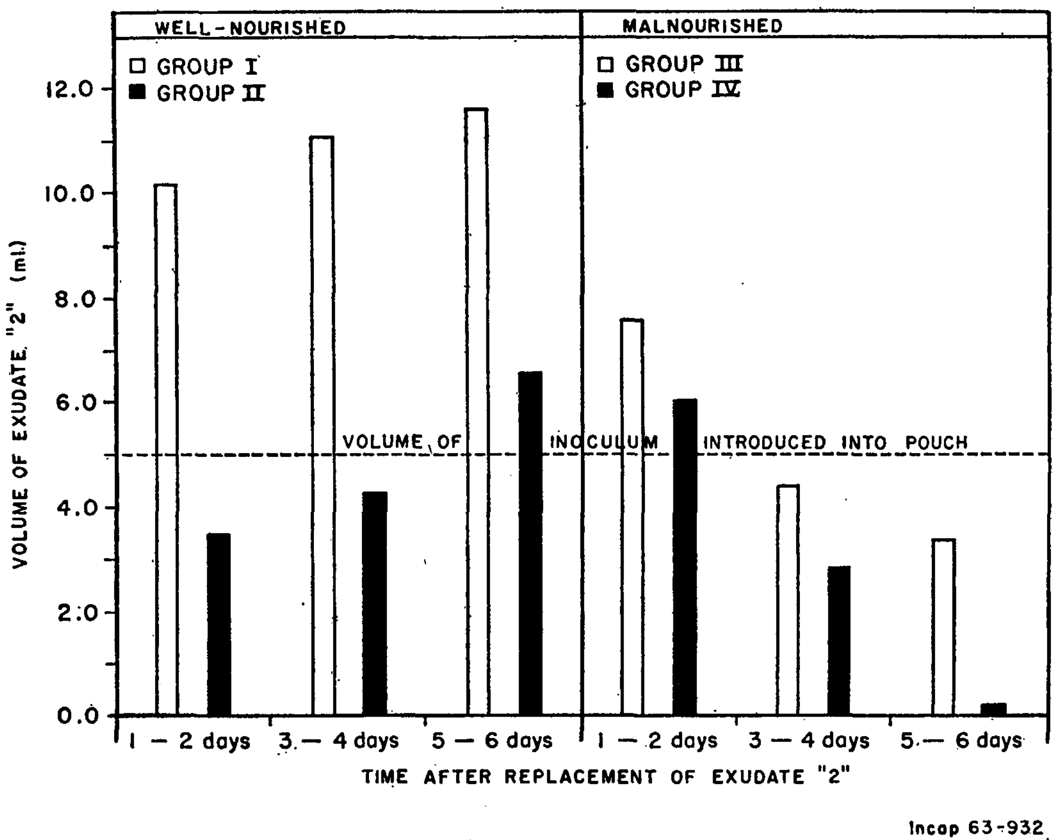

Fig. 3: Experiment D. Volume of exudate "2" harvested at various times during the experiment.

but subsequently, they were thickly purulent and creamy yellow.

Six out of 8 group II pouches from experiment C and 6/7 from experiment D yielded hemorrhagic exudates "2". In this group, hemorrhagic exudate formation initiated by croton oil apparently continued after the injection of saline-dextran. One pouch from experiment C contained no exudate "2" while the other had only traces of oil; in experiment D, one pouch yielded a clear, straw-coloured exudate "2". Six out of 8 group IV pouches from experiment C and 5/7 from experiment D contained exudates "2" that were purulent, but they were not as viscous or opaque as the exudates "2" from groups I and III. A number of these had a slightly pinkish tinge.

Bacterial content: Due to the limitations of the experimental conditions, exudates "1" were not cultured. All were hemorrhagic ,and did not appear to be grossly contaminated.

In experiment C, none of the exudates "2" from groups II and IV, injected with saline-dextran gave positive bacterial cultures. However, beta hemolytic streptococci were isolated from the inner scrapings of a pouch from an animal in group IV sacrificed 6 days after injection. This pouch contained no exudate "2". Streptobacillus-like organisms, which did not proliferate on blood agar, were seen in the smear of an exudate "2" from another animal in the same group examined 1 day after injection. In experiment D, strains of *Pseudomonas* were cultured from 1 exudate "2" from group IV harvested 1 day after injection, and from 1, harvested 3 days after; a strain of non-hemolytic streptococci was isolated from an exudate "2" harvested 2 days after injection.

Strains of bacteria with similar characteristics to the test organism were easily isolated, as pure cultures, from all exudates "2" of groups I and III of both experiments and from the inner scrapings of each of the pouches from experiment D examined 13 days after inoculation.

In both experiments, the trends shown by the concentration and total number of bacteria in exudates "2" were similar (Figures 4 and 5).

In experiment C (Fig. 4) there was an increase 1 day after inoculation in both dietary groups. Subsequent values for group I decreased, but group III increased 3 days after inoculation to values well above those for the corresponding group I

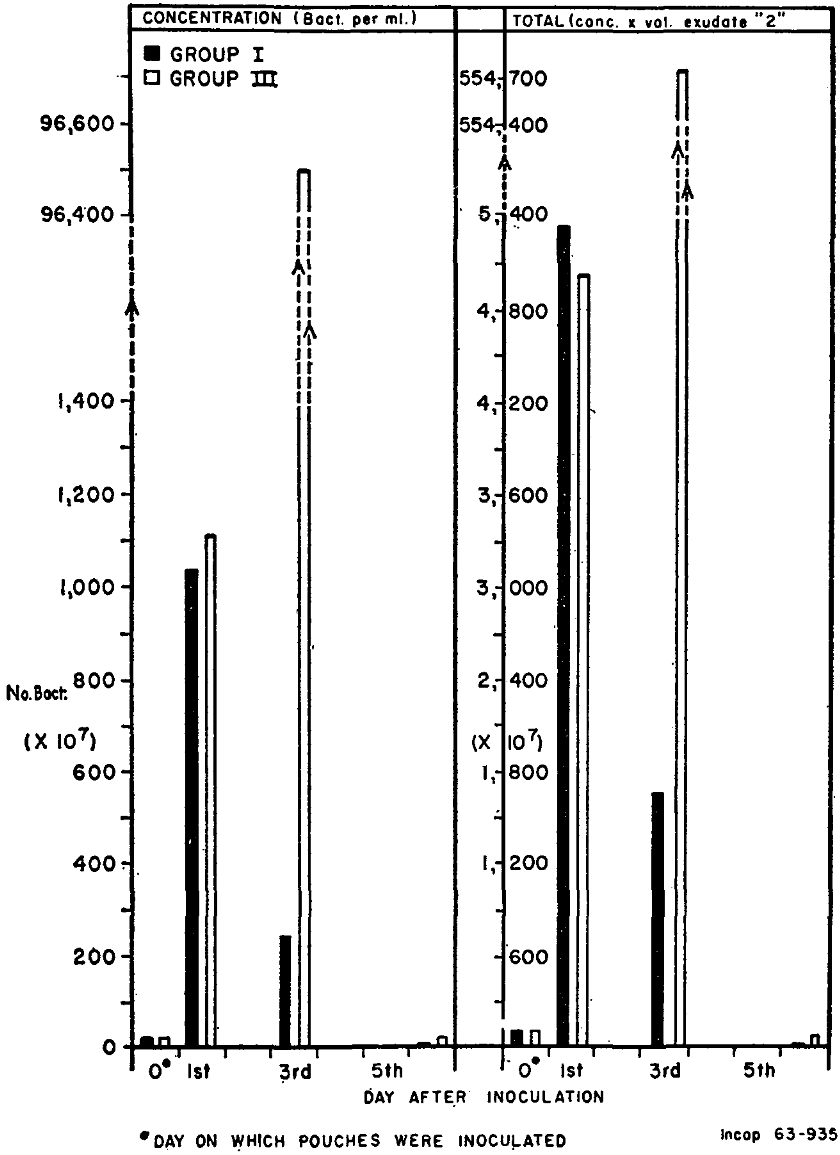

Fig. 4: Experiment C. Concentration and total number of bacteria in exudate "2".

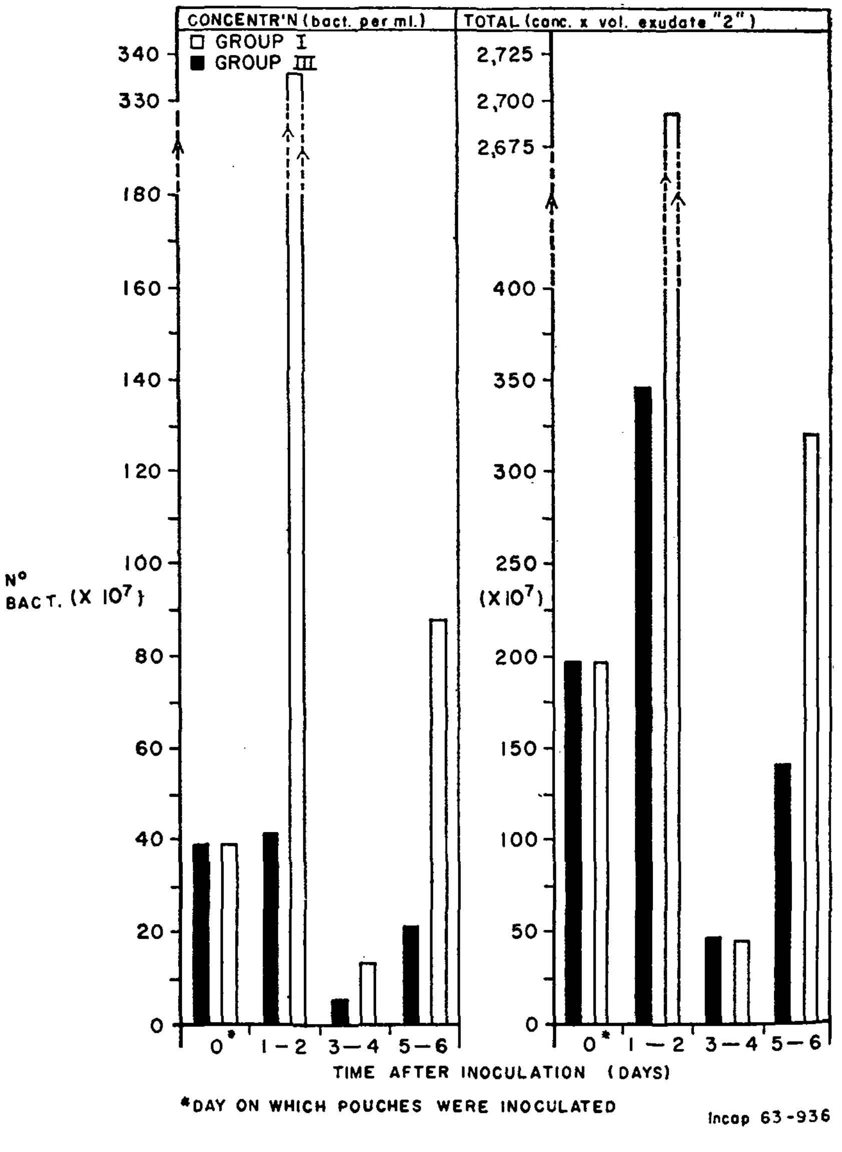

Fig. 5: Experiment D. Concentration and total number of bacteria in exudate "2".

animals. At 6 days, values for group III were only slightly higher than for group I. However, there was no statistically significant difference between the overall effects of the 2 diets, in the time trends of the 2 groups nor the interaction between diet x time after inoculation.

In experiment D, rats sacrificed 13 days after inoculation did not yield exudate "2" on which bacterial counts could be made. In general, the mean concentration and total number of bacteria for group III was greater than for group I (Fig. 5). The difference between the effects of the 2 diets on both concentration and total number of bacteria, and the overall time trend for each of these indices were significant ($P=0.05$ in each instance). No explanation can be offered for the secondary rise in the 5 to 6 day period. However, it is interesting to note that both this and the initial increase in the 1 to 2 day period were considerably greater for group III than for group I. This difference resulted in a significant time x diet interaction ($P=0.05$) for the total number of bacteria.

The bacterial counts for exudate "2" from experiment D were generally lower than for experiment C. The use of 1% croton oil, whose ability to stimulate exudate formation was superior in experiment D than in experiment C, as mentioned earlier, may have influenced this. Even though the croton oil exudate was removed at the time of inoculation, the mechanisms for the mobilization of tissue and blood cells and fluid at the site of inoculation, and the operation of other undefined defense mechanisms were already active and may have been responsible for a more rapid control of the infection. It is also possible that the 1 day difference in the time at which inoculation was performed in each experiment, 5 days after the preparation of the pouch in experiment C and 6 days after in experiment D, when superimposed on other differences in experimental conditions may have had some influence.

Cellularity: The concentration of nucleated cells in each smear of exudate "2" relative to the remaining smears increased with time in groups I and III (Table I); group III showed a greater increase than group I probably because of the more severe infection.

As to be expected, smears from groups II and IV whose pouches were not experimentally inoculated contained only a few nucleated cells. However, because of the high number

TABLE No. 1

MEAN CELLULARITY, BACTERIAL CONCENTRATION AND PHAGOCYTIC ACTIVITY OF NEUTROPHILS (% CONTAINING RECOGNIZABLE BACTERIA) IN EXUDATE "2" SMEARS

Experiment	Group	Day after inoculation	n	Cellularity	Bacteria	% Activity ± s. e.
C	I	1	4	+	3+	90.2 ± 4.0
		3	4	2+	2+	73.5 ± 8.9
		6	3	3+	+	33.7 ± 1.9
	III	1	4	2+	3+	85.6 ± 4.5
		3	3	4+	4+	62.0 ± 26.4
		6	5	3+	2+	54.0 ± 12.0
D	I	1 - 2	4	+	3+	86.0 ± 4.1
		3 - 4	4	2+	2+	61.2 ± 15.2
		5 - 6	4	3+	2+	68.5 ± 7.8
	III	1 - 2	4	2+	5+	89.5 ± 1.5
		3 - 4	4	3+	3+	72.2 ± 11.2
		5 - 6	4	4+	3+	70.2 ± 19.7

n = number of observations s. e. = standard error of accidental pouch infections in group IV of experiment D, more nucleated cells were seen in the smears from this group than in the other groups injected with saline-dextran.

Cellular composition: The cytological characteristics of exudates "2", determined by microscopic examination of smears are in accord with their previously described gross characteristics. In both experiments, neutrophils were the predominant cells in groups I and III. Smears from groups II and IV contained a lower proportion of neutrophils; group IV contained more than group II. Secondary infections and the possibility of a different type of reaction to the injection of saline-dextran probably contributed to this. In general, the relative concentration of neutrophils tended to increase with time. Erythrocytes were the predominant cells in only group II of both experiments. They were either absent or present in relatively low concentrations in the other groups. When present, there was a tendency for their concentration to decrease with time.

Bacteria in exudate "2" smears: All of the coverslip preparations from groups I and III in experiments C and D contained bacteria (Table No. 1) whose subjectively estimated concentrations paralleled the objective determinations made by agar pour plates (Figures 4 and 5). Occasionally, individual smears showed a higher concentration of bacteria than would be expected on the basis of the viable count. The presence of dead or dying organisms in the exudate "2" at the time of the preparation of the smear, and the difficulty in separating intracellularly located and extracellular clumps of bacteria in the exudate "2" prior to plate counting may have contributed to this discrepancy.

Bacteria were not seen in any of the smears from group II from either experiment. One group IV smear from experimet C contained a moderate number of extracellular organisms resembling streptobacilli; and 2 smears from group IV of experiment D contained rod-shaped organisms, mainly extracellular, of variable length; strains of *Pseudomonas* were isolated from the original exudates "2".

Phagocytic activity of neutrophils: In smears of exudate "2" from groups I and III prepared 1 day after inoculation in experiment C and 1 to 2 days after in experiment D, the number of bacteria present was large relative to the number of potential phagocytes, and the phagocytic activity was high in both groups (Table No. 1). There were still many extracellular bacteria. In both experiments, the phagocytic activity decreased with time. This decrease seemed greater in experiment C than in experiment D. In group I of experiment C, most of the bacteria were intracellular in the latter 2 periods. In contrast, there was a tendency for a large proportion of bacteria in the corresponding group III smears to remain extracellular in the presence of adequate numbers of phagocytic cells, suggesting that the activity of the cells from malnourished rats in this experiment decreased more than the concentration of bacteria. This effect was observed to a lesser degree in experiment D. The part played by other defense mechanisms such as antibody production and the bactericidal power of the pouch fluids in controlling the infection was not examined, but in view of the suggested trends show above, it should be borne in mind for future studies.

Characteristics of the Pouch. As in previous experiments (1) pouches from well-nourished rats were grossly better defined than those from malnourished rats, and on microscopic examination, the replacement of inflammatory cells by fibroblasts with accompanying collagen fibre formation was greater. In experiment D, the 2 pouches from groups I and III examined 13 days after inoculation were reduced to fibrotic nodules; the inflammatory reaction in these had completely subsided, and they appeared to consist essentially of scar tissue. The corresponding 2 pouches from groups II and IV tended to be nodular, but they were not as fibrotic as those inoculated with bacteria.

Histological appearance: The majority of rats in groups I and II of both experiments had compact inflammatory capsules. The pouches from groups III and IV were histologically less well-developed than those from groups I and II, and consisted essentially of thin capsules in which the connective tissue was loosely organized. Experimental and accidental infections were associated with thicker walled and more compact pouches. Young, active fibroblasts were abundant in the majority of pouches from all groups examined up to 6 days after the introduction of the test inoculum. Mononuclear cells seemed to persist longer and in higher concentrations in pouches from malnourished animals than in those from well-nourished. Collagen was not easily detected in pouches examined 1 day after the introduction of the test inoculum but it was apparent in older pouches. It was more evident in well-nourished groups. The difference between the 2 dietary groups increased with age of the pouch up to 5 to 6 days after the introduction of the test inoculum (Figure 6).

Pyogenic membrane formation was a prominent feature of 5/13 pouches from group III and 1/3 accidentally infected pouches from group IV of experiment C, and 6/13 and 2/4 pouches from each of these groups in experiment D. It usually involved only a part of the pouch wall, and appeared on the lumen surface of the capsule as a pseudomembrane of fibrin with enmeshed p.m.n. leucocytes; bacteria of other organisms were often present (Figure 7). Of the well-nourished inoculated and accidentally infected rats, 25 in all, only 1 from group I of experiment D exhibited pyogenic membrane formation. The difference between these frequencies was highly significant by the chi-square test ($P<0.01$).

All pouches inoculated with bacteria, except those reduced to fibrotic nodules, contained fibrinopurulent exudate within their lumen. Bacteria were easily seen in all except 2 from group I in experiment C. Bacterial invasion of the pouch wall was seen in only 3 pouches from experimentally inoculated rats, 1 from experiment C and 2 from experiment D; all were from group III. Fibrinopurulent exudate was present in the lumen of 1 group II and 2 group IV pouches from experiment C and 5 group IV pouches from experiment D.

Micro-organisms whose characteristics are described below were seen in the sections of all of these except 1 of the group IV pouches from experiment C and 2 from experiment D. Beta-hemolytic streptococci were isolated from the inner scrapings of the former. One of the latter contained a purulent exudate "2" from which a strain of *Pseudomonas* was isolated; the other was not found to be accidentally infected by the type of exudate "2" harvested, by culture on bacteriological media, nor by microscopic examination of exudate "2" smears or pouch sections.

Histology of Other Tissues. The most consistent finding on histological examination of other tissues was cloudy swelling of the parenchymal cells of the kidneys of all 4 groups in both experiments, examined up to 6 days after the introduction of the test inoculum. This type of alteration, essentially localized in the cells of the renal tubules, has been described previously as a consequence of the injection of dextran (6, 11, 12). It was not detected in the kidneys of animals examined 13 days after replacement of the test inoculum. Other workers have found that such changes are transient and do not lead to renal dysfunction (11-13).

Accidental Infections. Accidental infections were detected in a total of 1/15 group II rats (all from experiment C) and 7/15 group IV rats (3 from experiment C and 4 from experiment D). The difference between these frequencies was highly significant by the chi-square test ($P<0.01$). Beta-hemolytic streptococci were isolated from the pouch of 1 group IV rat from experiment C, while strains of *Pseudomonas* were isolated from purulent exudates "2" from 2 group IV rats of experiment D, and non-hemolytic streptococci from another. No isolations were made from the remaining accidentally infected pouches. However, coccus-like organisms were seen in the pouch section of a group II rat from experiment C; and fungal-like organisms with septate hyphae extending into the pouch lumen were observed in 1 group IV pouch from each experiment (Figure 8). Streptobacillus-like organisms were seen in the exudate "2" smear from a group IV rat in experiment C; fibrinopurulent exudate was not detected in the pouch section of this animal.

DISCUSSION

Interference with the local inflammatory response as a result of endocrine disturbances similar to those associated with malnutrition in humans (2, 14-17), or as a consequence of other physiological disturbances accompanying nutritional deficiency, was probably responsible for the finding that the pouch exudates of malnourished rats contained a higher concentration and total number of experimentally inoculated bacteria than the pouch exudates of well-nourished rats. In malnourished animals, a deficiency in the tissue fluids, cellular elements and other undefined factors that serve to control infection at the site of inoculation would allw the infecting organisms to become established in the pouch and multiply more rapidly than in well-nourished animals, especially in the

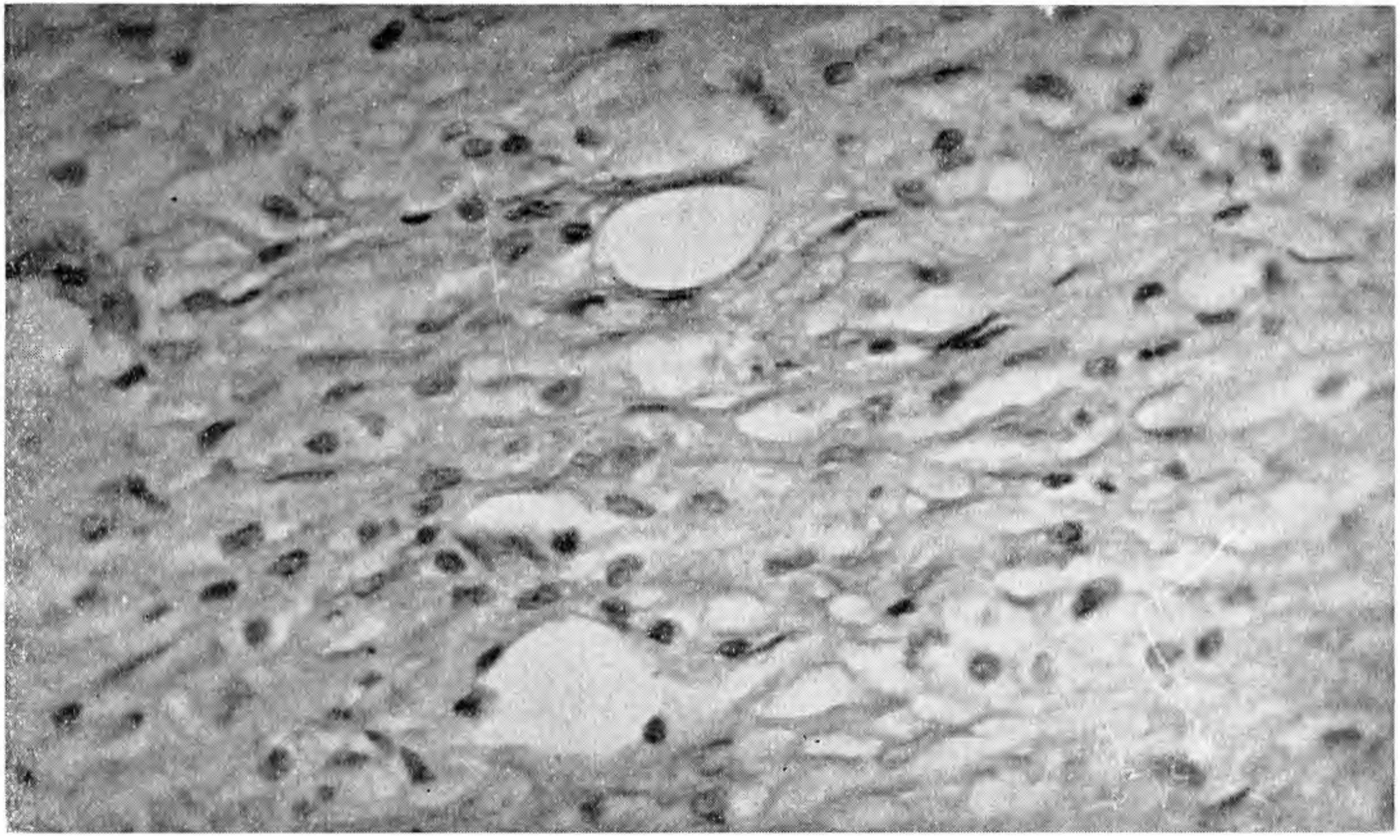

Figura 6a

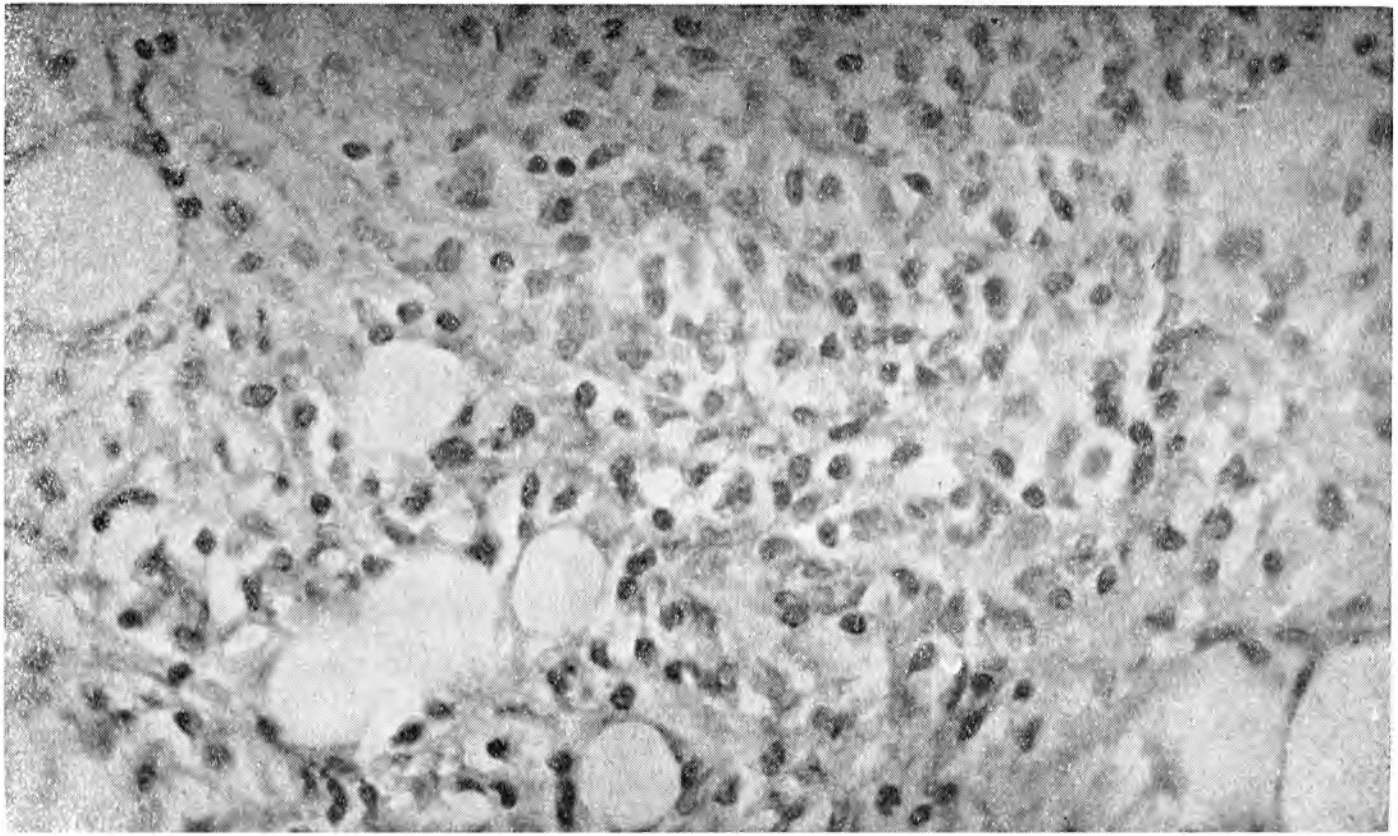

Figura 6b

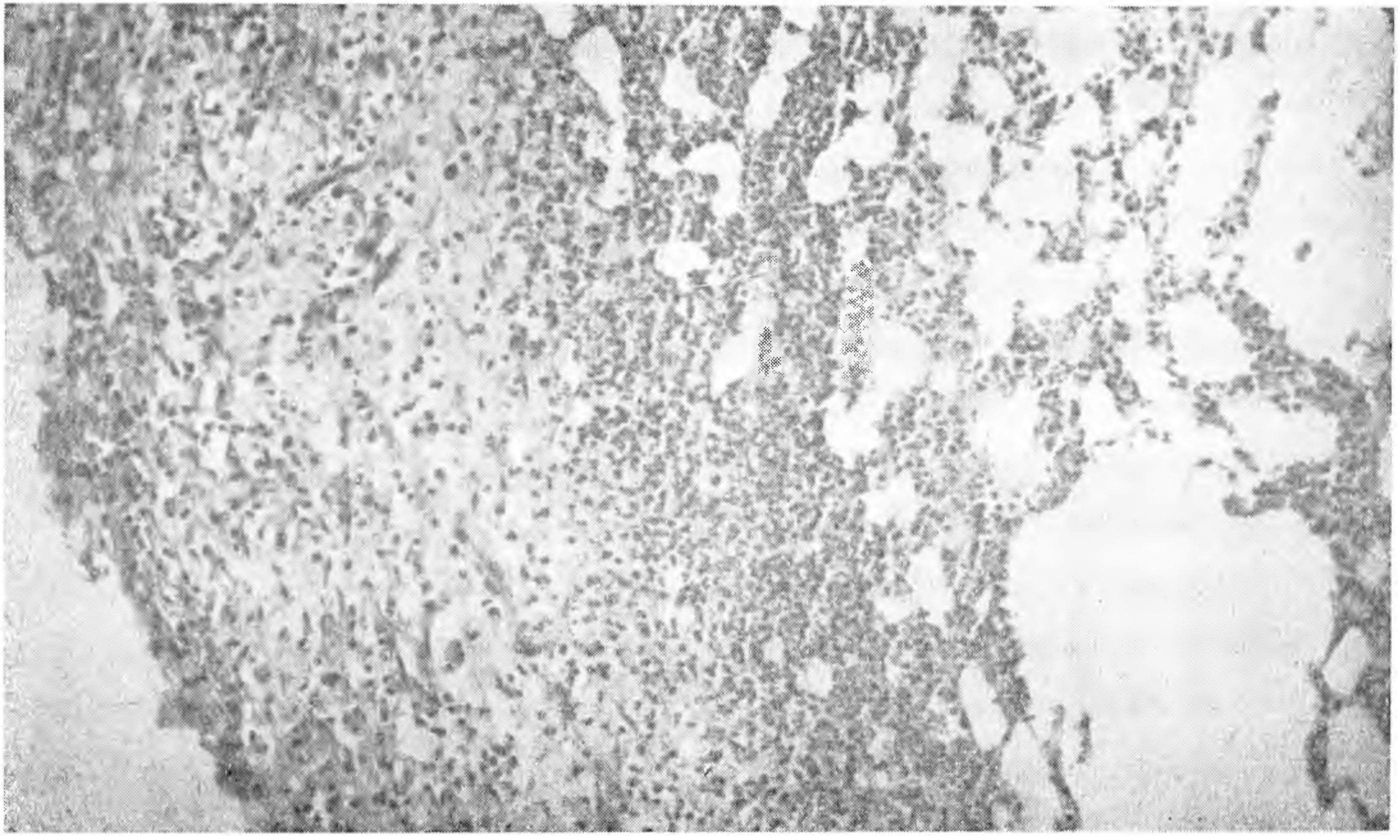

Figura 7a

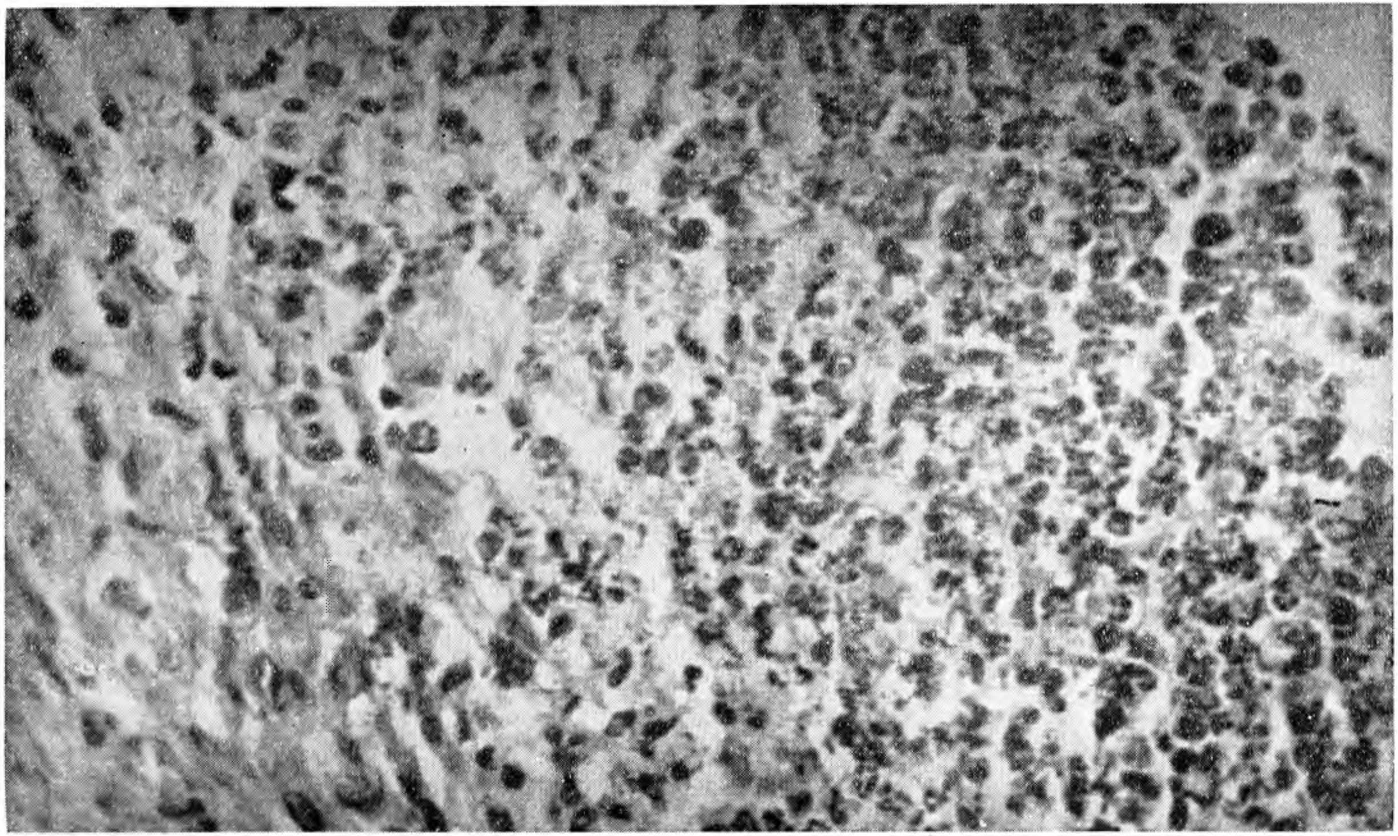

Figura 7b

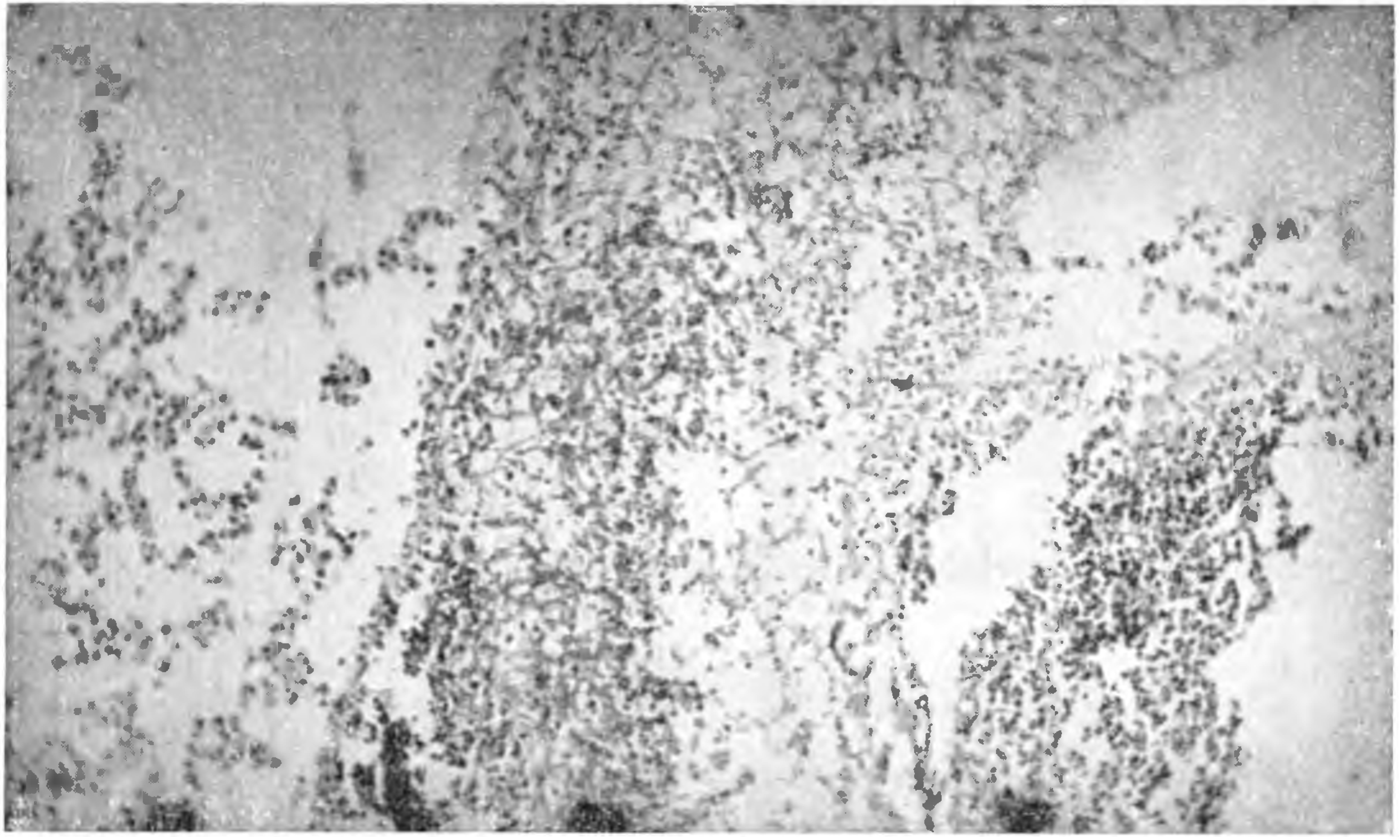

Figura 8a

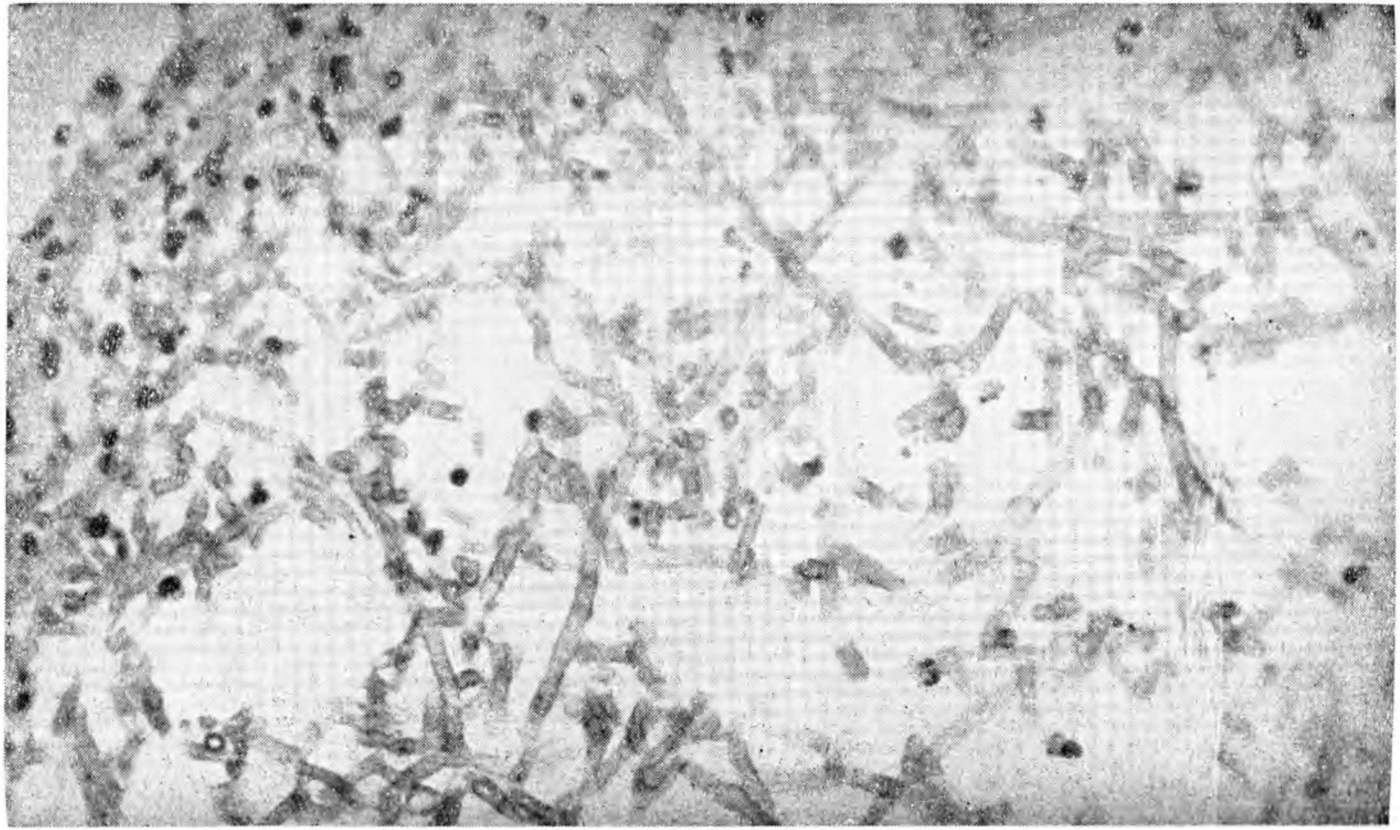

Figura 8b

Fig. 6: Experiment D. Histological appearance (hematoxylin and eosin, obj. 45, oc. 10) of pouch wall of well-nourished (group I) rat (Fig. 6a) and a malnourished (group III) rat (Fig. 6b) sacrificed in 5 to 6 day period after inoculation. Note abundance of collagen in Fig. 6a in contrast to Fig. 6b which has a predominance or mononuclear cells.

Fig. 7: Experiment D. Fig. 7a. Low power (hematoxylin and eosin, obj. 10, oc. 10) appearance of pyogenic membrane of pouch wall from a malnourished rat (group III), sacrificed in 5 to 6 day period after inoculation.
Fig. 7b. High power (hematoxylin and eosin, obj. 45, oc. 10) appearance of same wall.

Fig. 8: Experiment D. Fig. 8a. Low power appearance (hematoxylin and eosin, obj 10, oc. 10) of fungal-like mycelia seen in pouch section of malnourished rat (group IV) sacrificed in 5 to 6 day period after injection of saline + dextran. Fig. 8b. High power (hematoxylin and eosin, obj. 45, oc. 10) appearance of same section.

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earlier stages of the infection. Decreased local defense probably also facilitated the bacterial invasion of the pouch wall in some of the malnourished rats. In experiment C, the number of bacteria in the pouches of malnourished rats had decreased by the last-day of the experiment to values that were only slightly higher than in the pouches of well-nourished animals. This suggests that in malnourished rats there is a lag rather than an overall loss in the ability to bring into operation the defense processes needed to handle local infection.

The predisposition of malnourished rats to pyogenic membrane formation may reflect a fundamental difference in the physiological mechanism required to handle infection in the pouch. The accumulation of large numbers of p.m.n. leucocytes possibly compensated either for a decrease in the bactericidal power of the exudative fluid as seen in the experiments of Guggenheim and Buechler (18), or for an inadequacy on the part of the cells themselves; or the cells may have served to wall off the infecting organisms.

As in previous experiments (1), infection in the pouches of malnourished rats was able to produce a local response with respect to the type and volume of exudates and the macroscopic and microscopic characteristics of the pouch. The significant difference found between the time trends within the 2 dietary groups and between the overall effects of the 2 diets on the volume of exudate "2" may be explained at least partially by a more rapid absorption of the inoculum introduced into the pouches of malnourished rats and a subsequently decreased production of exudate in these animals.

The height of the leucocytic response to the various stimuli employed in animals given the deficient diet, was equal to or greater than that shown by those fed a complete diet, and neutrophilia accompanied leucocytosis. This agrees with the results of earlier studies. The absence of a statistically significant difference between the effects of the 2 types of inocula, saline +dextran and bacteria+saline+dextran, may indicate that dextran is capable of stimulating leucopoiesis and the superimposition of its effect on that of croton oil resulted in a maximum response equal to that following inoculation of bacteria + dextran. It is of interest that dextran is serologically reactive and capable of stimulating cutaneous sensitivity and the production of preciptins (19-21). Moreover, it is a macromolecular substance and has been detected in widely scattered phagocytes of the reticuloendothelial system after intravenous injection (12, 22); concurrent proliferation of such cells has been observed (22). Localization of the dextran within the pouch of animals in the present experiments would tend to hinder its free passage to the reticuloendothelial tissues and thus stimulate the proliferation of more mobile phagocytic cells.

Further support for a synergistic relationship between malnutrition and infection is presented by the high proportion of accidental infections in the pouches of malnourished rats. A wide variety of agents were found to be responsible, and infection occurred in spite of the aseptic precautions taken during all the operative procedures. The accidental infections did not seem to affect the systemic reaction as a whole, but the local response was heightened in a manner similar to that described in previous studies (1).

RESUMEN

Efecto de la desnutrición sobre la respuesta inflamatoria. Como lo presenta el desarrollo de la bolsa granulomatosa de la rata después de la inoculación con bacterias.

Los experimentos de los que aquí se informa describen el efecto del estado nutricional del huésped sobre la inflamación y la respuesta del tejido local de la rata después de la inoculación de S. aureus dentro de la bolsa granulomatosa. Se encontraron concentraciones más altas de los organismos a prueba en el exudado de la bolsa de aquellos animales ali-

mentados con una dieta deficiente, conteniendo de 3 - 4% de proteína, que en la de aquellos a quienes se les dio una ración completa, conteniendo aproximadamente 28% de proteína. También se observó invasión bacteriana de la pared de la bolsa en 3 ratas desnutridas, pero ello no se encontró en ninguna de las ratas bien alimentadas. Estas observaciones se consideraron ser posibles consecuencias de la respuesta inflamatoria deprimida encontrada en ratas desnutridas.

La formación de membrana piogénica se encontró en una proporción significativamente más alta en las ratas desnutridas infectadas que en aquellas bien alimentadas. Esto podría ser un reflejo de la diferencia fundamental del proceso fisiológico requerido por la rata desnutrida para defenderse de la infección en la bolsa. Aún más, una proporción significativamente más alta de las ratas desnutridas se infectaron accidentalmente con una amplia variedad de microorganismos. Sin embargo, no se encontró diferencia significativa en la respuesta sistemática de los dos grupos dietéticos a los diferentes tipos de inóculo usado.

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