Morphopathology of the Adenohypophysis of Chickens in Shock Induced by Escherichia coli

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SUMMARY. We studied modifications in the adenohypophysis of chickens subjected to experimental septic shock by repeated intraperitoneal inoculations with *Escherichia coli* 026 B6. We observed vascular modifications characterized by capillary dilation and endothelial defects, together with marked perivascular edema and collagen fibers in the groups receiving the most inoculations. Similarly, there was a proliferation of mononuclear cells, belonging mainly to the mononuclear phagocyte system, and plasma cells and lymphocytes.

The lesions found in chickens receiving only one inoculation may be evidence of a morphopathological relationship between shock induced by *E. coli* and lesions that develop in the swollen-head syndrome.

RESUMEN. Morfopatología de la adenohipófisis de pollos bajo choque inducido por Escherichia coli.

Se estudiaron las modificaciones de la adenohipófisis en pollos sometidos a un choque séptico experimental mediante inoculaciones repetidas vía intraperitoneal de *Escherichia coli* 026 B6. Se observaron modificaciones vasculares caracterizadas por dilatación capilar y defectos endoteliales junto con edema perivascular y fibras de colágeno en los grupos que recibieron el máximo de inoculaciones. Así mismo, se observó una proliferación de células mononucleares pertenecientes al sistema fagocitario mononuclear, lo mismo que de células plasmáticas y linfocitos.

Las lesiones encontradas en los pollos que recibieron sólo una inoculación pueden evidenciar que exista una relación morfopatológica entre el choque inducido por *E. coli* y las lesiones que se desarrollan en el síndrome de la cabeza hinchada.

Over recent years, many studies have shown that endotoxins, including those of *Escherichia coli,* cause lesions of the vascular endothelium (1,2,4). Tissue changes, mainly of mesenchymatous structures undergoing shock (4,8,9) in highly vascularized organs, have been studied (4,8,9). For this reason, and because of its cephalic location, we used the adenohypophysis as a research model.

The present studies were conducted to determine the connection between *E. coli* and the pathogenesis of swollen-head syndrome in broilers.

MATERIALS AND METHODS

Chickens. Fifty 30-day-old male white leghorn chickens, free of infections and parasitic disease, were divided into five groups of 10 chickens each. In each group, seven chickens were inoculated with 1.5-ml doses of *E. coli* and three chickens served as controls. Group I received one inoculation, Group II received two inoculations, Group III received three, Group IV received four, and Group V received five. Repeat inoculations were done at 24-hour intervals. The chickens were sacrificed 24 hours after the final inoculation.

Temperature. Rectal temperature was taken 6, 12, and 24 hours after the final inoculation using an ICOMATIC precision thermometer.

Necropsy. Birds were decapitated, and samples of hypophysis and 1 ml of blood for leukocyte count were immediately collected from each bird. No macroscopic alterations were observed in any organ, except for marked cerebral edema.

Histopathological procedure. The samples were fixed in Sublimate Bouin of Hollander for structural examination and then embedded in paraffin. Afterwards they were cut in 3-to-4-μm sections and stained with Herlant's tetrachrome, hematoxylin-eosin, and periodic acid-Schiff.

For the ultrastructural examination, samples were embedded in 2% glutaraldehyde and fixed in 2% OsO_4. After washing and dehydration in increasing concentrations of acetone, they were treated with propylene oxide and epon-araldite and finally embedded in epon-araldite. Forty-nm sections were cut from the blocks with an LKB III ultramicrotome. Two percent uranyl acetate and lead citrate were used to contrast sections.

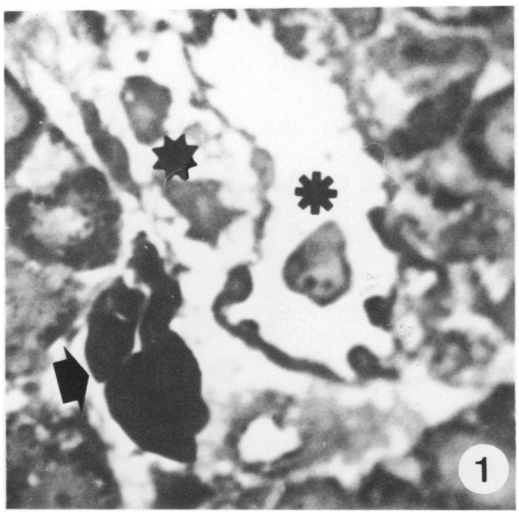

Fig. 1. Capillary dilation (*) with perivascular edema (star) and erythrocytes (→) observed in the first phase of the experiment.

RESULTS

Groups I and II exhibited vascular dilation, relaxation of the intercellular junctions of the endotheliocytes, simple necrosis of endotheliocytes, and extravasation of erythrocytes (Figs. 1, 2) towards the granular parenchyma, as well as marked perivascular edema in the whole organ.

Group III showed a marked increase in perivascular edema, together with microhemorrhages (Fig. 3) characterized by the presence of single erythrocytes between adenohypophysis parenchyma cells. Intravascular and perivascular proliferation of mononuclear cells began in this group (Figs. 4, 5). Lesions continued at the capillary level, characterized by the strong electrodensity of the cell organoids, endothelial defects (Fig. 6), and separation of the intercellular junctions (Fig. 7).

Capillary dilation persisted in groups IV and V, with endothelial defects and a decrease in perivascular edema and microhemorrhages. The proliferation of mononuclear cells, of both the lymphocyte type (Fig. 8) and those belonging to the mononuclear phagocyte system (Fig. 9), became more evident, with plasma cells (Fig. 10) in the perivascular space. A marked proliferation of fibers occurred, mainly collagen fibers and fibroblasts (Fig. 11).

The number of leukocytes and temperature are represented in Fig. 12.

DISCUSSION

Although the alterations occurring in the glandular component are extremely significant, at-

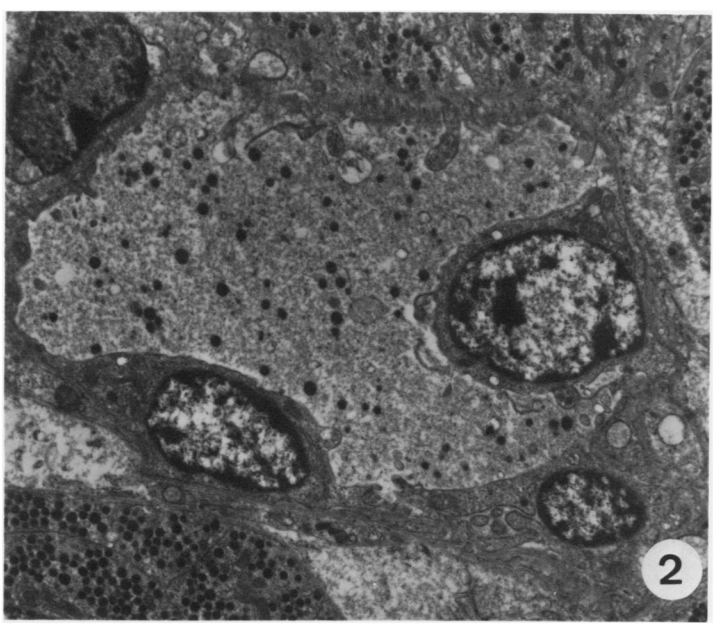

Fig. 2. Ultrastructural features of capillary dilation and perivascular edema corresponding to features illustrated in Fig. 1.

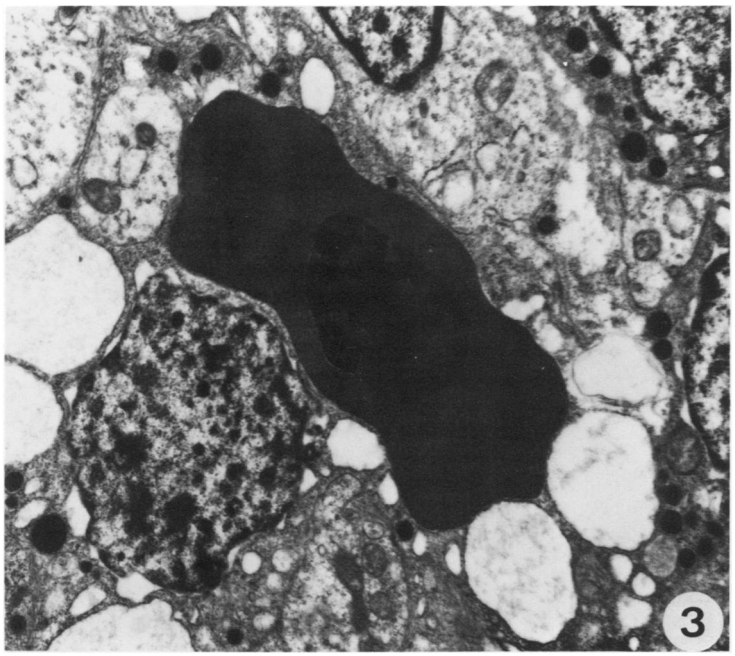

Fig. 3. Erythrocytes in the glandular parenchyma (microhemorrhage phenomenon), frequently found in the acute phase.

tention should be focused on lesions caused by shock, since these are primary lesions responsible for the respiratory insufficiency (3,4) that leads to other alterations.

Vascular lesions appeared in all experimental groups and were characterized by severe edema, microhemorrhages, and endothelial defects. In any septic shock (2,3,10), and particularly in those caused by *E. coli* endotoxins, there exists a primary phase of vascular reaction known as the "vascular syndrome" (11).

The marked perivascular edema observed in the adenohypophysis parenchyma in all experimental groups appears in the initial shock phase (8) and is mainly due to alterations of the vascular endothelium. Equally significant was the decrease in edema in Groups IV and V, coinciding with the leukocyte proliferation stimulated by the edema; this is a typical feature of all shocks (3). The decrease of the edema, coupled with the appearance of cell proliferation and collagen, is typical of chronic shock processes (6) due to the repeated action of the etiologic agent, which seems to activate lymphatic stimulating factors, a phenomenon detected in individuals subjected to chronic shock processes (2,3,6).

The perivascular edema is accelerated by the

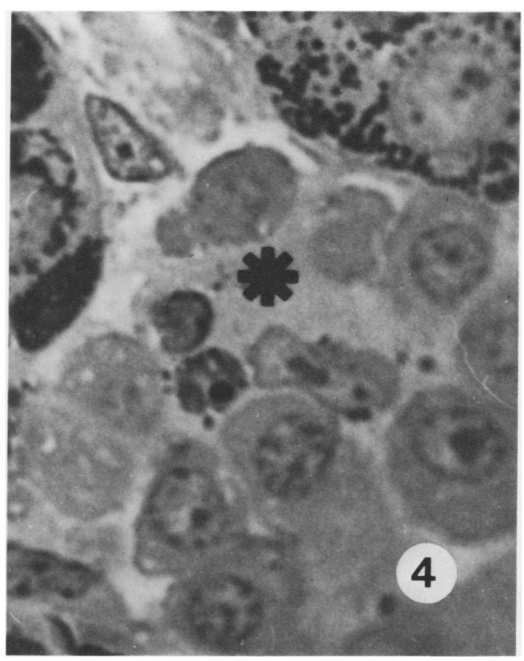

Fig. 4. Leukocyte proliferation (*) present in the third and subsequent experimental groups. Leukocyte presence appears to be involved in the immunological phenomenon of shock.

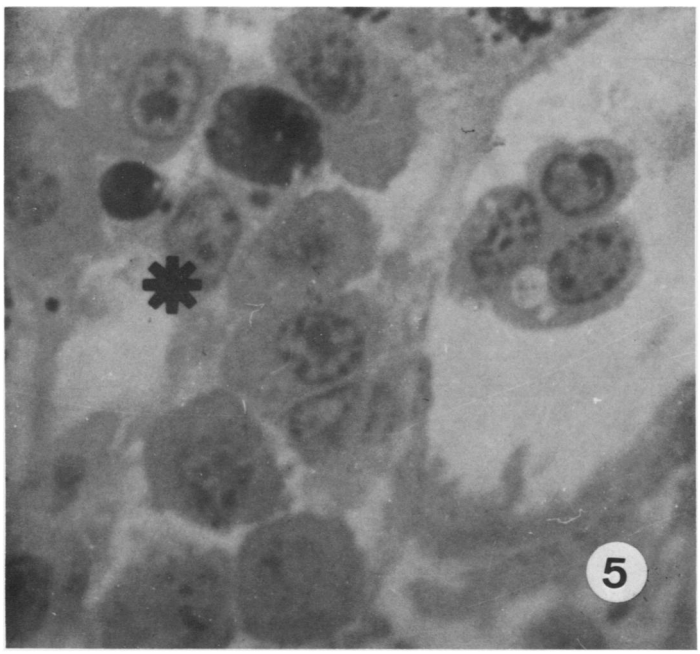

Fig. 5. Leukocyte proliferation (*) present in the third and subsequent experimental groups is apparently involved in the immunological phenomenon of shock.

endothelial modifications observed in the first groups but was most evident in Group III. Such endothelial modifications are characterized by the simple necrosis and relaxation of intercellular junctions common in the first phases of shock (4,7).

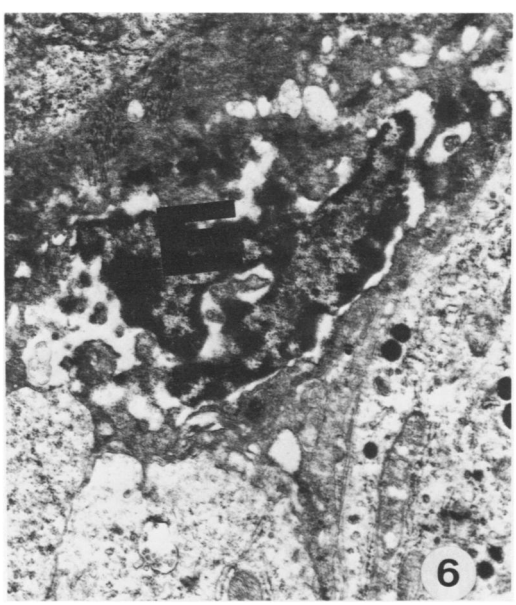

Fig. 6. Necrosis of an endotheliocyte (E), favoring the edema and hemorrhaging processes.

Similar alterations of glandular components have been reported (7,12) for the swollen-head syndrome, which is characterized by severe edema and microhemorrhages.

These vascular modifications were most marked in Group III, and we attribute this to an acute process of lesion formation in these animals, a mechanism for stimulating the still-insufficient lymphatic system (2,12).

All the alterations previously described give rise to microhemorrhage processes, single erythrocytes being observed in areas of cell necrosis or between normal glandular cells, extravasated by a process of diapedesis, which is typical in states of stress and acute shock (4). Similarly, we attribute the intensity of microhemorrhages in the later groups to two factors: first, the acute shock suffered by the chickens in the first group, and second, the morphopathologically chronic shock suffered by the chickens in later groups, particularly in Group V (2,3), which gives rise to a decrease in microhemorrhaging together with tissue-repairing processes.

Edema and cell proliferation, mainly of plasma cells, began in the third group and became more evident in later groups, probably owing to im-

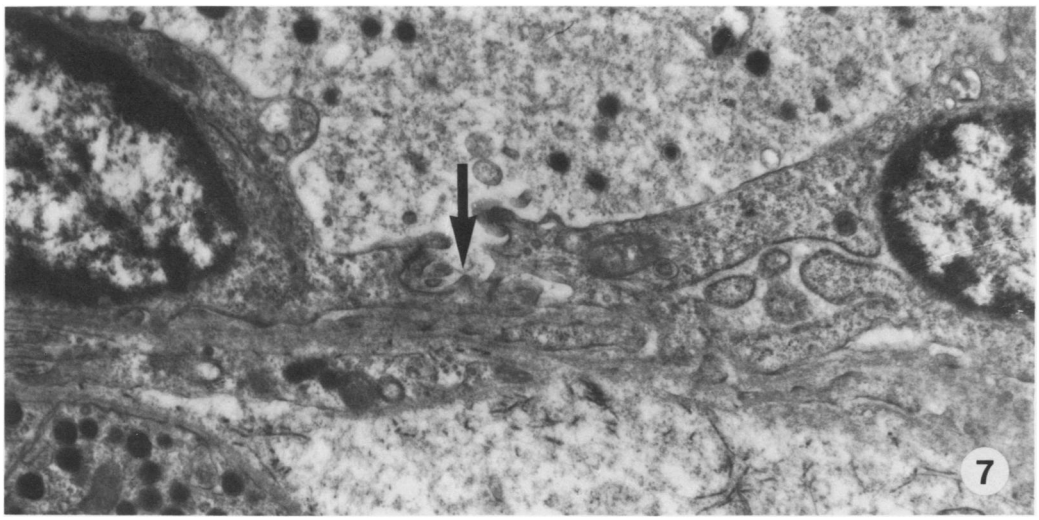

Fig. 7. Ultrastructural detail showing beginning intracellular separation (→) in a capillary. Perivascular edema is present.

munological processes that complicate the organism's response (3,4). Proliferation of plasma cells is also found in the swollen-head syndrome in broilers (7,12), in which it has also been related to immunological phenomena at advanced stages of that syndrome.

The existence in Groups IV and V of collagen fibers is due to processes occurring in the "vas-

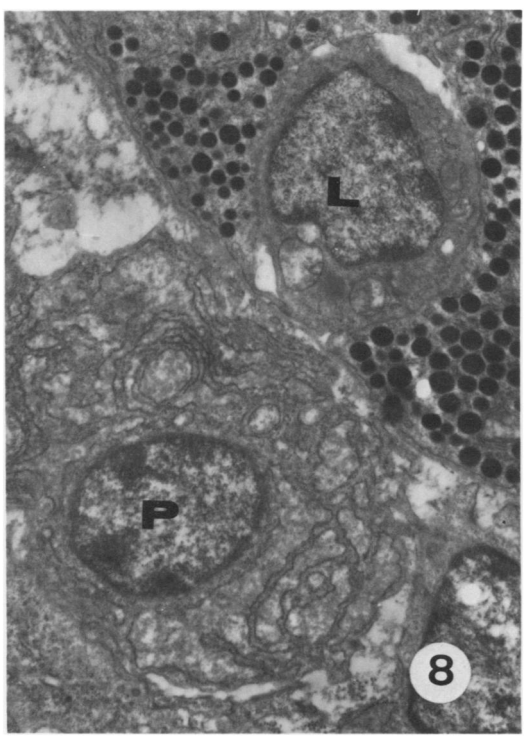

Fig. 8. Both plasma (P) and lymphocyte (L) cells proliferated in the fourth and fifth groups (chronic phase).

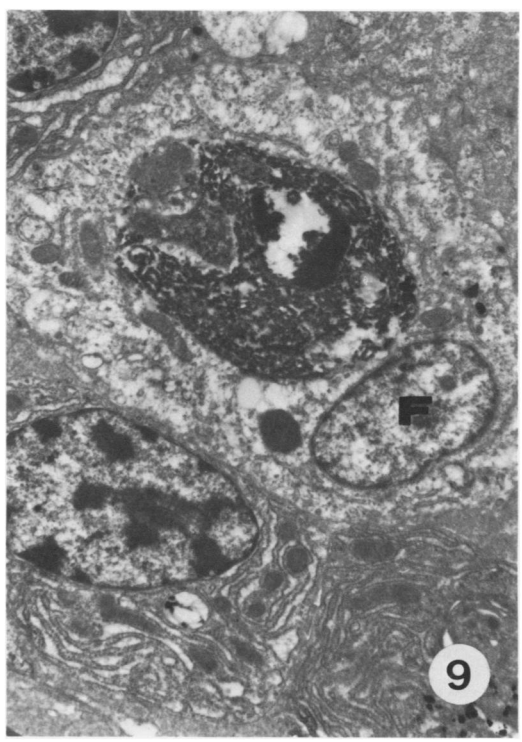

Fig. 9. Macrophage (F) ingesting a necrotic plasma cell. Both participate in defensive processes.

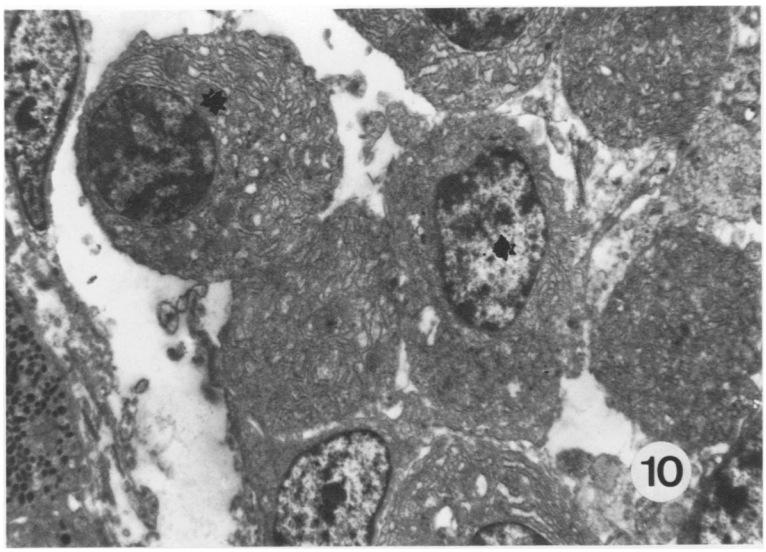

Fig. 10. Large numbers of plasma cells (*) are involved in the immunological phenomenon of shock in the second phase of the experiment.

cular syndrome" (1,5,8) in chronic restoration phases, and it indicates the point of irreversible damage in any shock process (8,9).

Analysis of body temperature showed that the chickens adapted their physiological constants to their new state of health, and yet lesions that could be termed chronic have been established, with irreversible alterations in the later experimental groups.

In any prolonged septic shock process, leu-

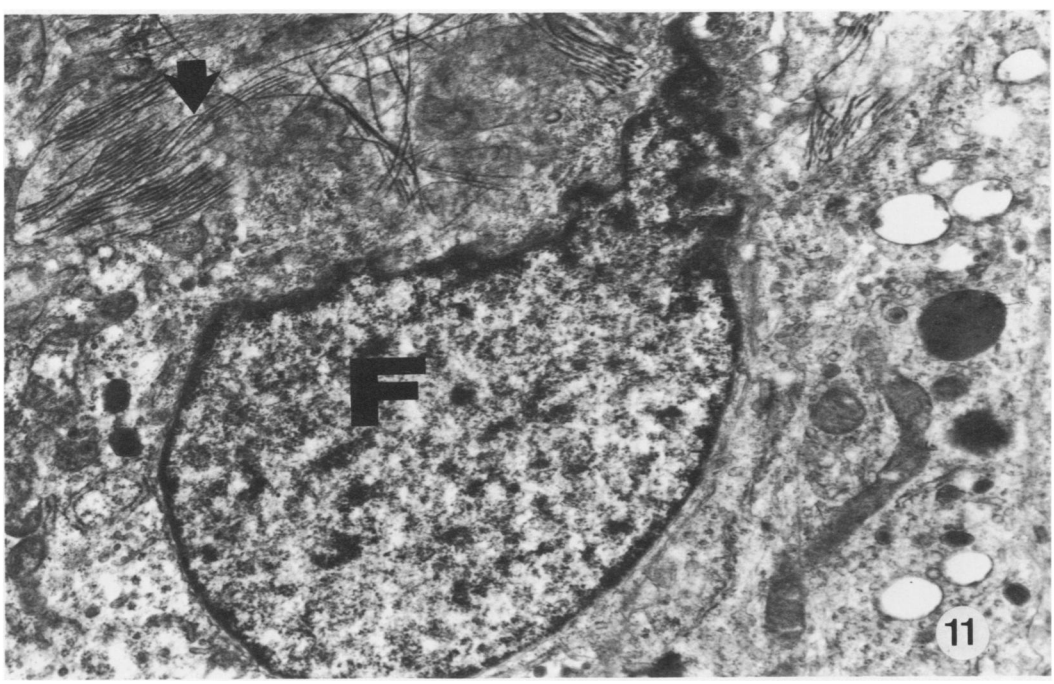

Fig. 11. The presence of fibroblasts (F) and collagen fibers (→) indicates the point of irreversible damage in chronic shock (chronic phase).

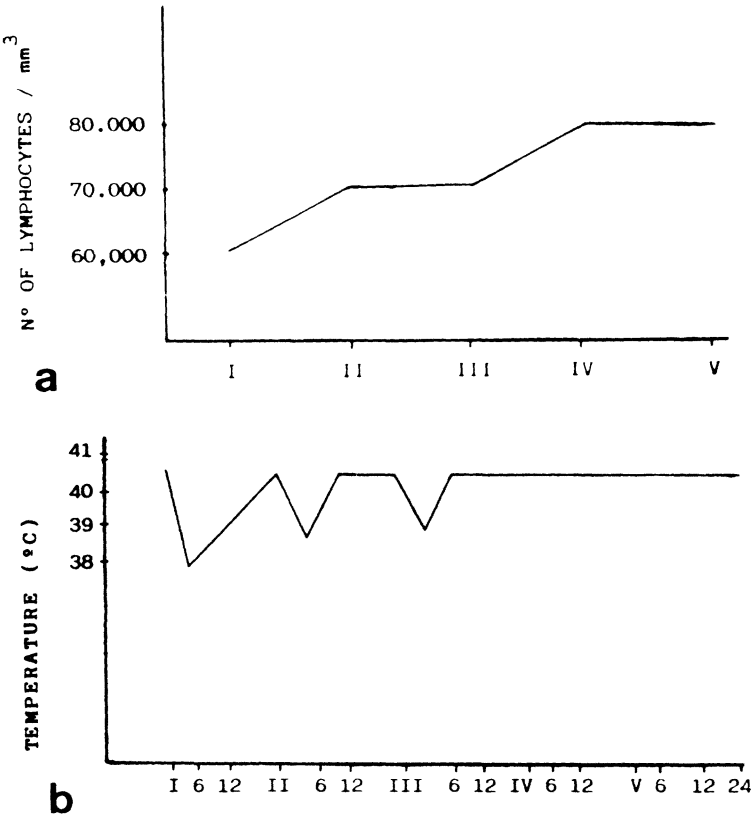

Fig. 12. Changes in lymphocytes 24 hours postinoculation (a) and temperature 6, 12, and 24 hours postinoculation (b) in the experimental groups.

kocytosis occurs, owing to the extravasation of lymphocytes and blastic bone marrow cells (3) into the vascular network.

The cycle of lesions throughout this experiment shows the adenohypophysis to be a model of shock, in which morphological alterations occur in two phases: acute and chronic. The former could be said to consist of the edematous lesions caused by the endotoxic action of *E. coli,* which are also observed in the swollen-head syndrome in broilers (7,12).

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