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EXPERIMENTAL REGENERATION OF THE LUNGS

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Following their observations on the time course of healing of lung wounds, many investigators have accepted the view that regeneration of the lung parenchyma with partial or even total resorption of the scar is a possibility [1-4]. Healing of lung wounds was associated with proliferation and migration of the epithelium of those bronchi and alveoli that are damaged as a result of wounding. The bronchial epithelium invades the zone of the defect and takes part in the formation of structures resembling alveoli [2, 12, 14]. It was postulated later that alveoli may also be formed from the alveolar passages in the antenatal period and soon after birth, in response to loss of part of the lung [6, 10, 15]. However, no convincing proof of the existence of this process has yet been obtained.

The aim of this investigation was to study regeneration an incised stab wound of the lung and to identify the most active cellular sources of regeneration.

EXPERIMENTAL METHOD

An aseptic wound of the lungs was produced in 150 male guinea pigs weighing 280-300 g at the age of 5-6 months under sterile conditions and under local anesthesia with 0.25% procaine solution, an incision through the skin, subcutaneous areolar tissue, and spinal and intercostal muscles 1-1.5 cm long was inflicted posteriorly on the right side in the 6th intercostal space. A stab wound of the diaphragmatic lobe of the right lung was then inflicted by puncture with a special scalpel fitted with a guard. The depth of the wound was about 10 mm and its width 5-6 mm. The animals were killed by decapitation after 10 min, 1 h, and 1-7 and 14 days. The lungs were quickly removed from the thorax and investigated by various methods. The role of cyclic AMP (cAMP) in the regulation of many cellular metabolic reactions justified the investigation of this system after lung damage. Fractions of cytoplasmic membranes were obtained from the guinea pigs' lungs. The number of β-adrenergic receptors was determined from the maximal binding of [^{3}H]-dihydroalprenol. Activity of adenylate cyclase (AC) was determined from the quantity of [^{32}P]-cAMP formed. The latter was investigated by the method in [13]. The protein concentration was determined as in [11]. The wound edges, the tissue surrounding the wound, and its symmetrical area of the

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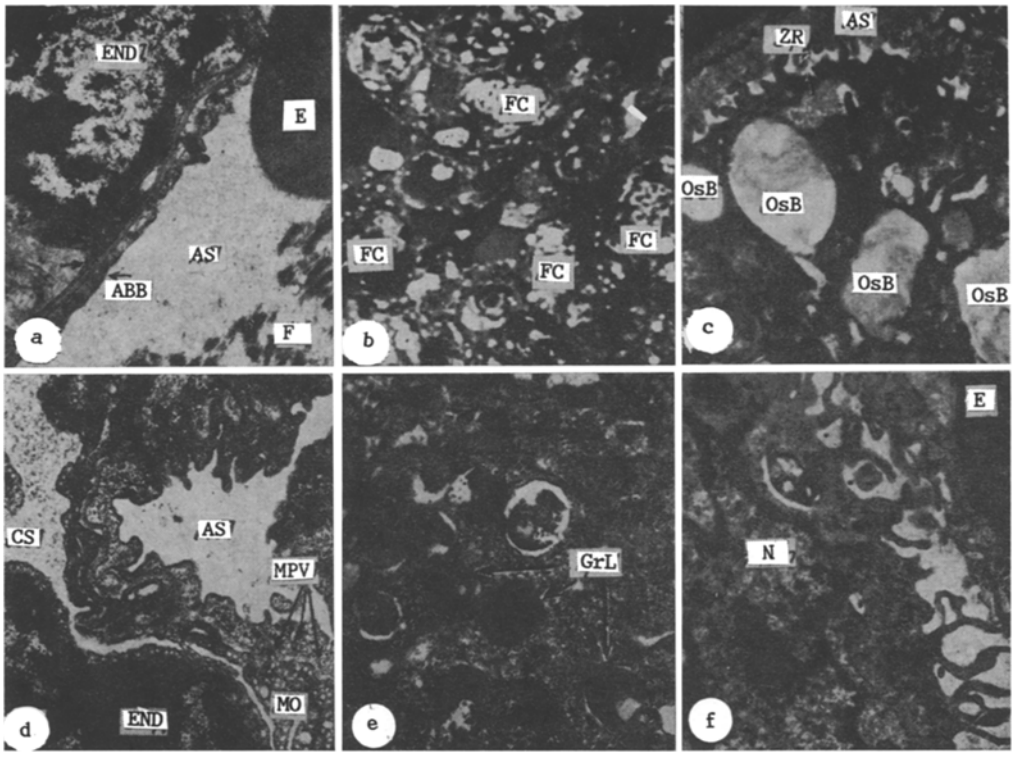

Fig. 1. 1-4 days after lung injury. a) Hemorrhage and fibrinous exudate in an alveolus. 10,000 ; AS) alveolar space, ABB) air-blood barrier, F) fibrin, END) endotheliocytes, E) erythrocytes; b) foam cells (FC). 400 ×; c) rarefaction of exudate in zone of plasmalemma of PN-II. 4000 ×. OsB) Osmiophilic body, ZR) zone of rarefaction; d) morphology of activity of endothelial cells in zone of lung injury. 6000 ×, CP) capillary space, MPV) micropinocytotic vesicles, MO) microscopic outgrowth; e) labrocyte in septal stroma of alveolus in zone of injury. 8000 ×. GRL) Granules of labrocyte; f) active macrophage in the same zone. 4000 ×. N) Nucleus of macrophage.

opposite lung were investigated histologically and electron-microscopically. Ultrathin sections were studied in the Tesla BS-500 electron microscope.

EXPERIMENTAL RESULTS

A fall of the cAMP level in the cell is a characteristic indicator of proliferative processes. The cAMP concentration depends on AC activity.

From the first few hours after wounding of the lung a progressive decrease took place in the number of β-receptors and the level of AC activity and, correspondingly, of the cAMP concentration, which fell to 50% of the control value on the 3rd-4th day. During healing of the lung wound these parameters gradually increased. By the 7th-14th days, they had regained their initial levels. The time course of changes in the concentration of components of the cAMP cycle, reflecting the character of regeneration in the lung wound, enabled the optimal times of the morphological investigation to be identified, namely: the 1st, 3rd, 4th, 7th, and 14th days.

Marked necrobiotic and necrotic changes with atelectasis and edema of the lung parenchyma and hemmorrhages into it predominated in the wound edges (Fig. 1a). Meanwhile the initial manifestation of compensatory repair reactions were observed. The number of alveolar macrophages was considerably increased. Neutrophilic leukocytes were present inside the capillaries. Many type II pneumocytes (PN-II) — so-called granule cells (Fig. 1b) with giant empty osmiophilic bodies or with the material in them partially preserved, began to appear. Their morphology indicates increased release of surfactant into the alveoli, thereby restoring their surface tension. Accordingly, an electron-translucent zone 20-30 nm wide appeared in the alveolar space, evidently as a result of the presence of surface-active

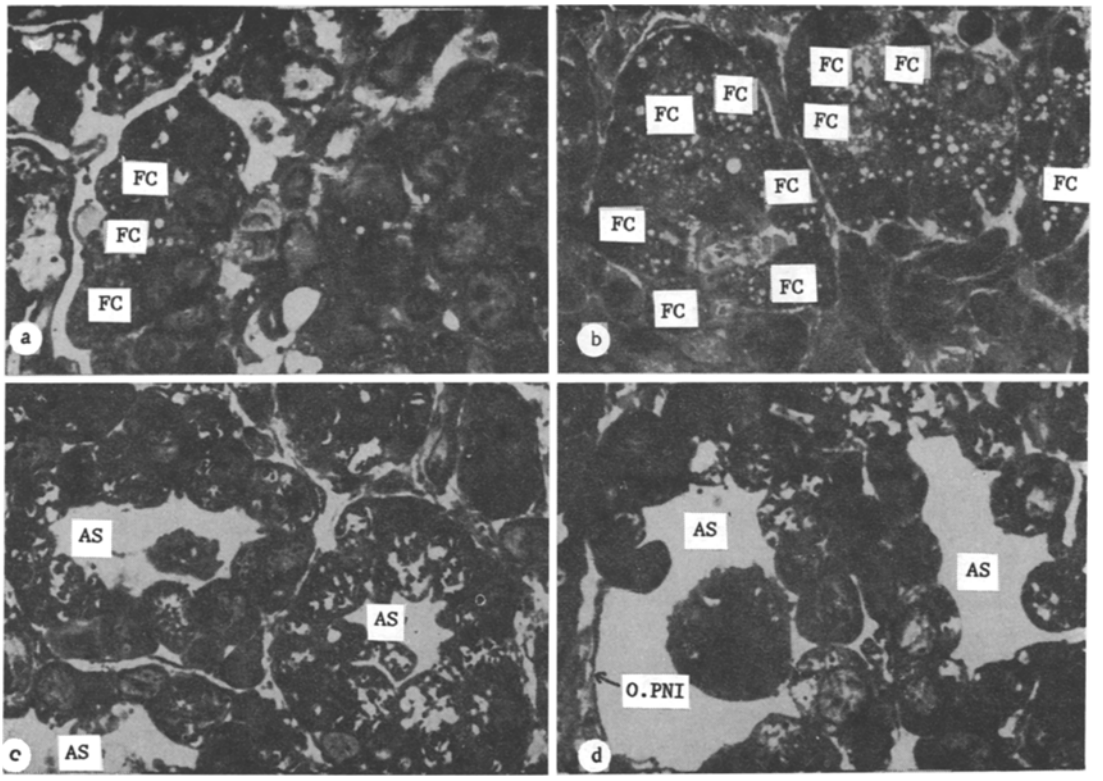

Fig. 2. Dynamics of formation of new alveoli in zone of damage to lung parenchyma. a) Bud formation from PN-II. 1000 x; b) Increase in foam content of PN-II and beginning of formation of alveolar lumen. 1000 x; c) Formation of alveolar lining by PN-II only. 1000 x; d) Appearance of outgrowths of PN-I in newly formed alveolus (0.PNI). 1000 x.

substances near PN-II (Fig. 1c). The number of polyribosomes and cisterns of the endoplasmic reticulum also was increased in cells of types I and II of the alveolar epithelium and also in the endotheliocytes. In the zone near the wound, signs of injury were rather less marked and compensatory and reparative reactions were more evident. In the opposite lung marked hypertrophy and hyperplasia of the organelles of PN-I and PN-II were observed, in agreement with results obtained previously [5, 6].

On the 3rd-4th day after the operation young granulation tissue formed in the wound edges. Around it, in the capillaries of the air-blood barrier, endotheliocytes formed microscopic outgrowths, often surrounding the capillary. Pinocytosis in the endothelium was intensified, the number of mitochondria increased, and indentation of the outlines of the nuclei and margination of the chromatin in them were distinctly seen (Fig. 1d). Single lymphocytes and labrocytes were present in the interalveolar septa (Fig. 1e). Atelectasis and dystelectasis of the alveoli still remained, with accumulation of transudate in them, although to a rather lesser degree than one day after wounding. Meanwhile in the alveoli still possessing a lumen, numerous alveolar macrophages containing erythrocytes and remnants of osmiophilic bodies could be detected (Fig. 1f). The number of PN-II was increased and their cytoplasm was rich in mitochondria and osmiophilic bodies, containing amorphous material. The number of ribosomes and mitochondria was increased in PN-I and the endotheliocytes and the cisterns of the lamellar complex were dilated, evidence of predominance of intracellular regeneration. On the whole at this time more marked ultrastructural changes were visible in all cells of the lung parenchyma compared with the previous times. Changes in the zone near the wound and in the opposite lung were on a smaller scale than at the previous time of the experiment.

On the 7th-8th day after lung injury mature granulation tissue was observed to be formed in the wound edges. The number of atelectases and dystelectases in the surrounding parenchyma was distinctly reduced. Hardly any transudate was left in the alveoli. The macrophages had become smaller. The number of lymphocytes and labrocytes was increased, possibly evidence of

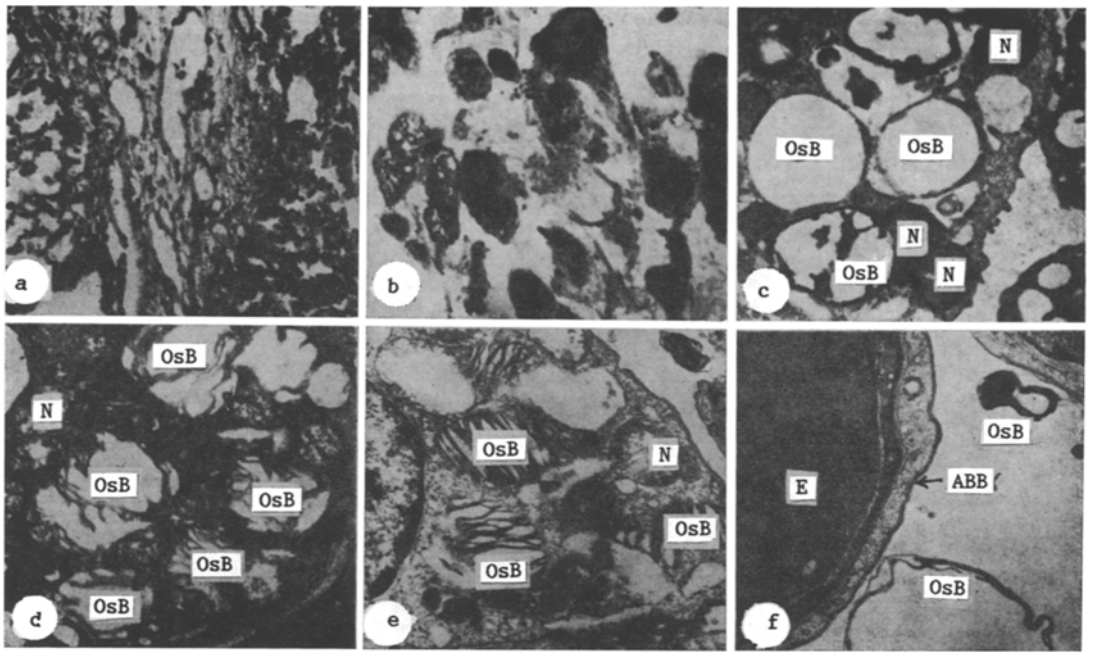

Fig. 3. Healing of lung wound and time course of regeneration of osmiophilic bodies. a) Granulation tissue in zone of former wound. 400 x; b) agglomeration of active fibroblasts in zone of granulation tissue. 1000 ×; c) osmiophilic bodies one day after lung damage. 8000 ×; d) the same, after 7 days; e) the same after 14 days; f) single lamellae of osmiophilic bodies in alveolar space 21 days after operation. 10000 ×.

preservation of the inflammatory response. Meanwhile cellular cooperation of this kind combined with fibroblasts is known to play a regulatory role in processes of cell proliferation and differentiation [9]. Hardly any degenerative changes could be seen in the pneumocytes and endotheliocytes, but they did show signs of mitosis, and evidence of intracellular regeneration [7, 8]. However, because of the increase in volume of the endotheliocytes, basement membranes, and PN-I the air blood barrier was rather thicker than before. Processes of intracellular regeneration continued in PN-II with an increase in the number of osmiophilic bodies and in the number of lamellae contained in them. During this period agglomerations of PN-II appeared in the form of buds, consisting of 4 to 6, or sometimes 15-20 PN-II, and this was the initial stage of formation of new alveoli (Fig. 2a, b). In semithin sections at these times and later, areas of rarefaction could be distinguished in the center of the agglomerations of PN-II, and the arrangement of PN-II in a single row on the basement membrane also was noted (Fig. 2c). In our opinion this marks the next phase of formation of new alveoli. In some newly formed alveoli, one-third to one-quarter of their circumference was free from PN-II, evidently due to transformation of PN-II into PN-I (Fig. 2d). Our own observations and the data in the literature indicate the similarity of processes involved in the formation of new alveoli in the antenatal period and after lung damage [5, 10, 15].

On the 14th day of the experiment active fibroblasts, often forming agglomerations, and also separate fibrous structures could be seen in the region of the former wound in histological and semithin sections in the mature granulation tissue (Fig. 3a, b). However, no sign of development of a mature scar could be observed at these times.

Characteristic changes were found in the osiophilic bodies of PN-II. On the 1st-3rd days they increased in size and their content became less electron-dense, on the 7th-8th days lamellae appeared in them, and on the 14th day the number of parallel lamellae became much greater (Fig. 3c, d, e), indicating not only the continued formation of surfactants, but also its accumulation in the cells. Regeneration of the wound ended with the formation of new alveoli, lined with PN-II containing many osmiophilic bodies. Restoration of alveoli in the zone surrounding the former would took place through hyperfunction of PN-II, forming surfactant intensively (Fig. 3f). The possibility cannot be ruled out that reduction of the granulation tissue in the wound region takes place under these conditions.

Regeneration of the injured lung thus takes place through cellular and intracellular

mechanisms of lung tissue restoration. In all probability PN-II is the cambial cell with the highest proliferative potential, and providing the basis for the formation of new alveoli and restoration of the air-blood barrier.

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REACTIONS OF SKIN MICROVESSELS TO LIMB BLOOD FLOW CHANGES STUDIED BY PHOTOPLETHYSMOGRAPHY AND LASER DOPPLER FLOW

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KEY WORDS: photoplethysmography; laser doppler flowmetry; microvessels; noradrenalin; occlusion

An important theoretical and practical aspect of the problem of arterial hypertension is the study of the functional state of the microcirculatory bed, which may be evaluated on the basis of the character of responses of the microvessels to physiological factors: vasoactive substances, a change of temperature or arterial blood pressure, and so on [2-4]. Our own investigations have demonstrated altered vascular reactions of the skin to noradrenalin (NA) in patients with arterial hypertension of varied etiology: essential hypertension (EH) and pheochromocytoma [2, 6]. Vascular responses were assessed by photoplethysmography (PPG), based on measurements of the intensity of reflected monosomatic light with a wavelength of 580 nm, transformed into an electrical signal. The new, noninvasive method of studying the microcirculation, namely laser doppler flowmetry (LDF), is based on the principle of measurement of the frequency shift of monochromatic red light of wavelengths 633 nm (generated by a helium-neo laser), arising during reflection from moving blood cells [5]. This method differs

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