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Pleural Mesotheliomas and Asbestos Pleurisies due to Environmental Asbestos Exposure in Turkey: An Analysis of 120 Cases*

Dr. Y. İzzettin Barış**

Introduction

Asbestos is the general term given to a group of minerals which are fibrous in character and resistant to high temperatures, the two qualities on which more than 1000 kinds of industrial uses depend. The most commercial fibers are chrysotile (white), crocidolite (blue), amosite (brown) and anthopyllite.

Although asbestos is known since ancient times, its commercial exploitation was modest until the late nineteenth century, when as a result of the industrial revolution, the need arose to develop the means for insulation of the steam engine.[1] Between 1887 and 1967, asbestos production and use increased from 50 tons to 4 million tons per year. Asbestos is widely used industrially, especially in fire-proofing, the reinforcement of cement in building materials and manufacture of brake linings and other friction materials.

The adverse effects of asbestos on health were first observed in the early 1900's and today it is recognized that occupational, para-occupational and environmental exposure to asbestos may lead to many pat-

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hologic conditions. Such conditions include fibrosis of the lung and pleura; and neoplasms of the lung, pleura, peritoneum, and gastrointestinal tract,[2-6] and, possibly, the larynx,[7-10] ovary,[11] and then breast.[12]

It can be drawn from the records of the Institute of Mineral Research and Exploration (M.T.A.) of Turkey that certain regions in Turkey including Eskişehir, Sivas, Bursa, İzmir, Uşak, Aydın, Adana, Kastamonu, Kars, Çankırı, Elazığ, Diyarbakır and others, are rich in asbestos.[13] In Turkey, the literature concerning asbestos was first confined to case reports of mesothelioma.[14-23] The fact that asbestos would be considerably hazardous to health and create a health problem for this country was first pointed out by the studies of the Department of Chest Diseases of Hacettepe Medical School, carried out in asbestos mines and mills around Mihalliççik and Sivas in 1972; thus pleural calcifications and pulmonary fibrosis were given emphasis.[24] Afterwards these studies were continued in the villages of Mihalliççik and Maden to examine the effects of environmental exposure to asbestos of the peasants in rural regions.[25-28] Similar studies have been carried out by Yazıcıoğlu et al from the Medical Faculty of Diyarbakır University during the years 1973-1975 in south-eastern part of Turkey.[29-31] Çelikoğlu et al[32] reported that they found asbestos bodies in the lungs of the 25 per cent, of the consecutively performed autopsies in Istanbul.

The study of Barış et al concerning the epidemy of mesothelioma in Karain/Ürgüp, once again brought up the consideration that asbestos exposure in Turkey might be an important health problem, especially from the point of view of cancer.[33] This recent study points out to the fact that further clinical, epidemiological and experimental studies on asbestos are required in Turkey; the following clinical study may well be an introduction to such efforts.

Material and Method

37 cases from the village of Karain previously reported constitute the first group of the patients examined.[33] The second group is made up of 15 patients, who are not from this village, examined at the Faculty of Medicine of Hacettepe University. The third group includes the previously reported 22 cases from the Medical Faculty of Ankara University,[23] 15 cases from the Atatürk Sanatorium of Keçiören;[15-17] 9 cases from Süreyyapaşa Sanatorium[21]; 8 cases from Gülhane Military Medical Academy,[19] and 15 other cases summing up to 68. The total number of patients examined was 120.

The files of the patients who were admitted to the hospitals in Ankara were examined individually. Data on their names, age, sex, place of birth, place of residence, profession, past and family history, social and environmental details, the patients' symptoms and their duration, physical and x-ray findings of the lungs, laboratory results (erythrocyte sedimentation rate, PPD, chemical, bacteriological and cytological examination of the pleural fluid and sputum), initial diagnosis, methods of diagnosis, pathology of specimens, clinical course management were drawn from the files.

While establishing the diagnosis of mesothelioma, metastatic cancer of the pleura, primary and metastatic lung cancer, tuberculous pleurisy, collagen diseases, pulmonary infarction and different causes of tansuda were considered in the differential diagnosis. Suspected cases of mesothelioma and asbestos pleurisies were not included in the study.

All of the patients examined at Hacettepe were questioned after their occupational and environmental exposure to asbestos; and the soil which they used for toilet, stucco and a special sweet was geologically examined.

Pleural biopsies from 5 patients were examined by electron microscope. Mineralogical examination with the electron microscope and x-ray diffraction in pleural biopsy specimens were made in two patients.

Results

108 of the 120 patients were diagnosed as malignant pleural mesothelioma, 9 as asbestos pleurisy, and 3 as benign pleural mesothelioma. Seven of the patients with asbestos pleurisy were diagnosed at Hacettepe, two at other hospitals; using tissue diagnosis, ruling out other causes of pleural disease and a follow up period of minimum 2 years. Five of these patients were from the village of Karain.

Table I shows the distribution of the ages and sex of the 120 patients examined.

TABLO I

AGE AND SEX OF THE PATIENTS

	The Youngest Patient	The Oldest Patient	Average Age	Total
Male	15	71	42.7	72
Female	12	69	50.6	48
			Total	120

The following map (Figure 1) shows the regions where the patients were born, raised and lived throughout their lives.

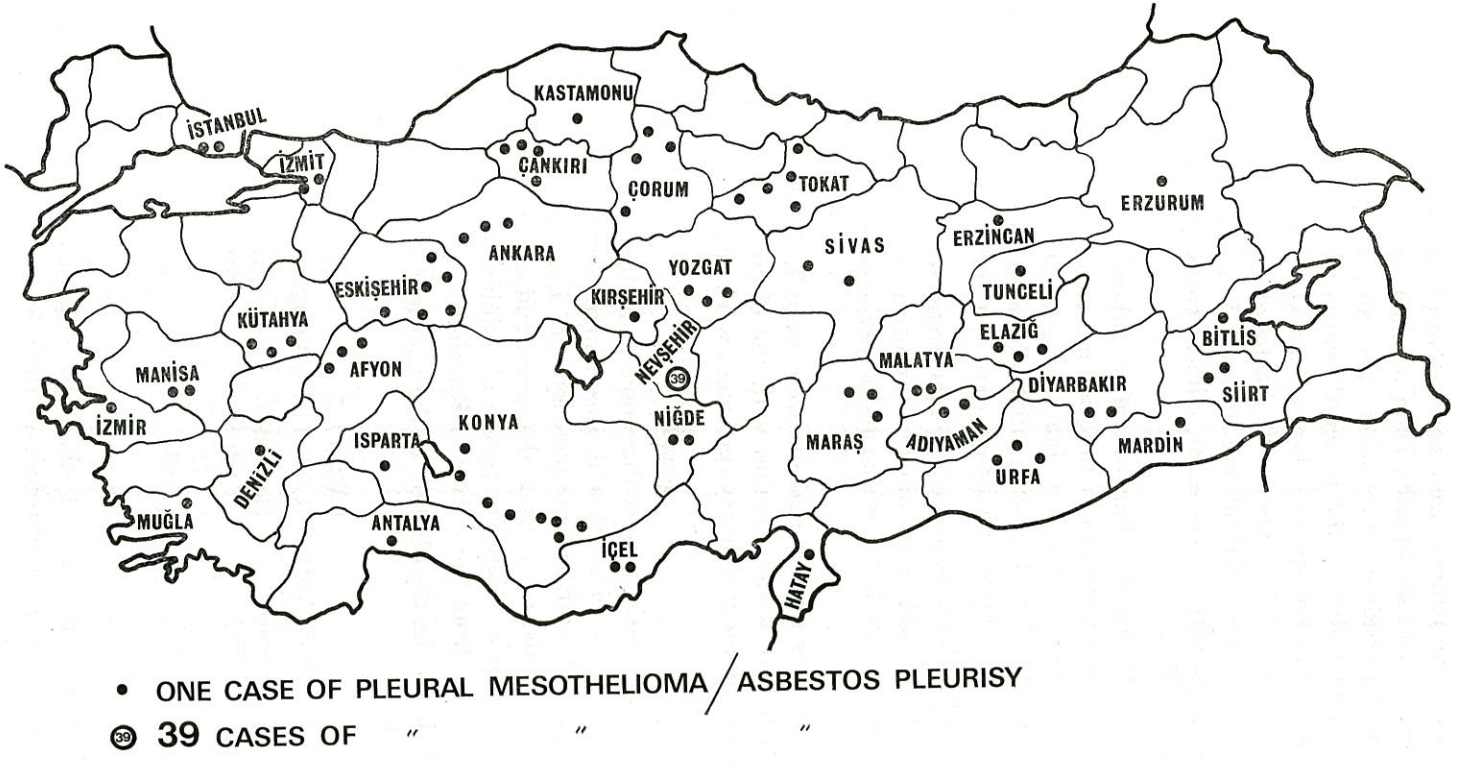

Figure 1

Distribution of the 120 persons with malignant pleural mesothelioma, asbestos pleurisy and benign mesothelioma according to place of residence.

Only 2 of the 120 patients had an occupational exposure to asbestos. One of them was from İzmit and had worked in the automobile industry, the other was from Istanbul and was employed as a construction worker.

Social and environmental conditions from the point of view of asbestos were very much the same, excluding the two workers mentioned above. All of the remaining 118 patients were peasants, making a living as farmers. All of the Hacettepe patients had used "white stucco", put this specific kind of soil in their sweets and revealed that they had also used it for toilet needs during their childhood.

The main symptoms of the patients with malignant pleural mesothelioma and asbestos pleurisy were pain in the chest and dyspnea. One of the patients from Karain diagnosed as asbestos pleurisy, revealed that he had been treated with a diagnosis of left pleurisy 25 years ago. The chest x-ray of this patient taken in 1971 and 1975 are shown in Figures 2 and 3.

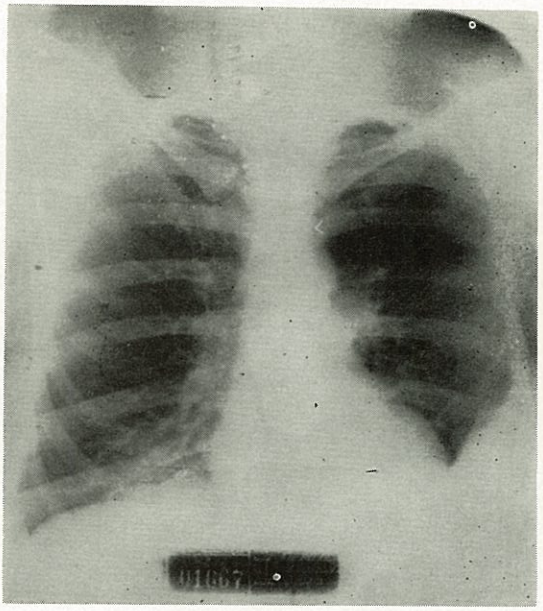

Figure 2
Chest x-ray of a patient from Karain taken in 1971. Diagnosis: Asbestos pleurisy.

All of the patients from Karain have a family history of mesothelioma and/or neoplastic disease (Figure 4). None of the 15 patients from other regions who were examined at Hacettepe showed such conditions.

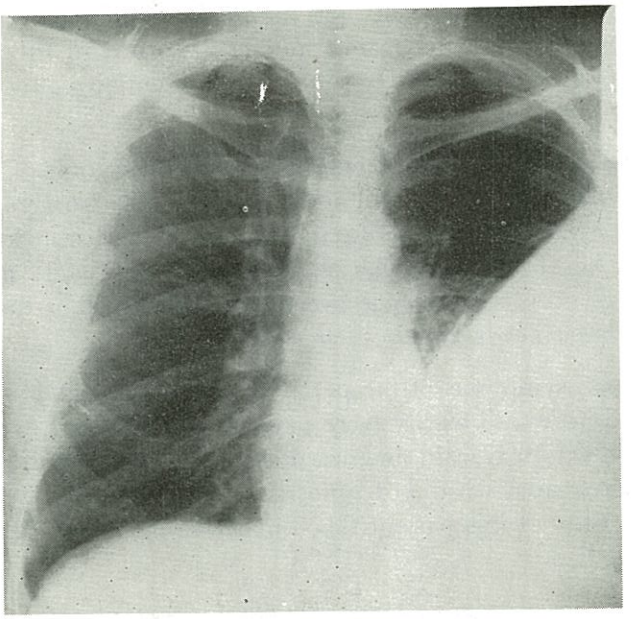

Figure 3
Second chest x-ray of the same patient taken in 1975.

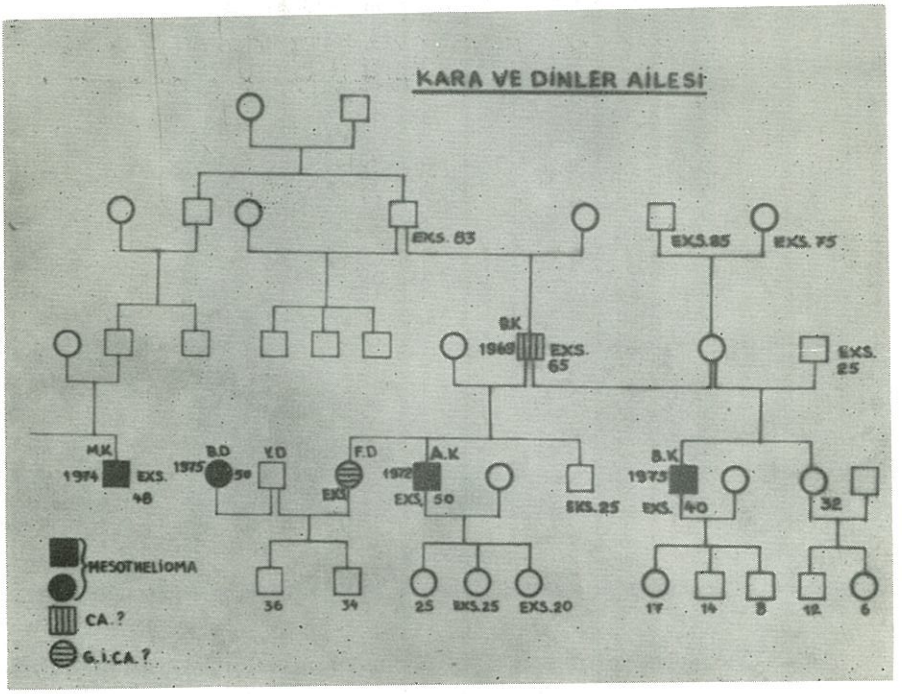

Figure 4
Family tree of the cases form Karain. Kara and Dinler Family.

The physical examination of the patients with malignant pleural mesothelioma and asbestos pleurisy revealed the findings of pleural thickening or effusion. Only one case of malign mesothelioma had Horner's syndrome. Vena cava superior obstruction was seen in a patient with asbestos pleurisy.

Radiological examination showed pleural thickening, and pleuropulmonary opacities and pleural effusion. Four cases had pleural calcifications suggestive of asbestos exposure, along with mesothelioma findings. Figure 5 shows one of these cases.

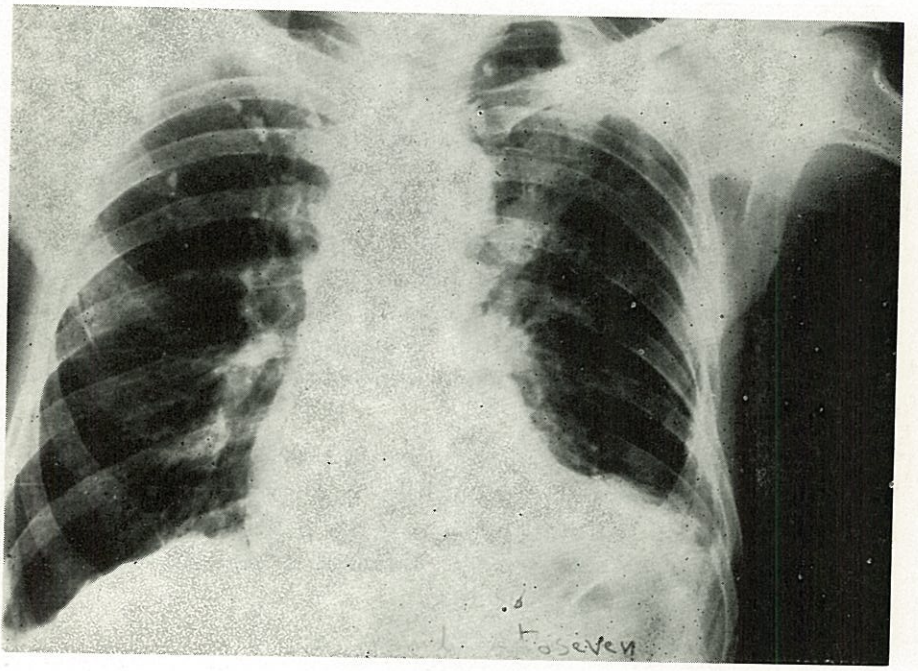

Figure 5

Chest x-ray of a patient from Karain diagnosed as malignant pleural mesothelioma. Showing diaphragmatic calcification on the right side, and peripheral opacity with pleural thickening on the left side.

Among the 108 malignant pleural mesothelioma 55 were right sided, 53 were left sided. X-ray findings in benign pleural mesothelioma were suggestive of hydatid cyst. There were pleural calcifications in one of these patients.

In malignant pleural mesothelioma patients, the minimal erythrocyte sedimentation rate (E.S.R.) was 4 mm in the first hour, the maximal E.S.R. was 115 mm. in the first hour and the average 55.8 mm.

Only three out of nine patients with asbestos pleurisy were tested for E.S.R., and these showed 31 - 41-60 mm/hr. Twelve malignant mesothelioma patients were tested for PPD and two gave negative, five positive results. Table II shows the results of cytologic examinations of pleural fluids and the sputum.

TABLO II

CYTOLOGIC EXAMINATIONS OF THE PLEURAL FLUIDS AND SPUTUM

	Class I	Class II	Class III	Class IV	Class V
Pleural fluid	–	1	2	1	2
Sputum	2	6	1	–	–

The protein level of the pleural fluid was measured in 21 cases, the minimal was 1 gm. per cent, the maximal 6.2. gm. per cent, with the average being 3.5 gm. per cent. Pleural fluid glycose was maximum 130 mg %, minimum 15 mg % and the average 54.2 mg. %. Two cases of malignant mesothelioma had a pleural fluid glycose of 15 mg., the other two 25 mg and the remaining two cases 25-50 mg per cent. In two patients with malignant pleural mesothelioma, both the pleural fluid and the blood glycose content were found to be very low and these patients had hypoglycemic attacks.

The macroscopic appearance of the pleural fluid showed polymorphism. It was serofibrinous in 18 cases, sero-haemorrhagic in 11 cases and haemorrhagic in 7. In a great majority of the patients the macroscopic appearance turned to haemorrhagic although it was sero-fibrinous or sero-haemorrhagic in the beginning.

Table III shows methods of tissue diagnosis.

TABLO III

METHODS OF TISSUE DIAGNOSIS IN 75 CASES

	Chronic fibrous pleuritis	Mesothelioma		Insufficient	Total
		Benign	Malign		
Pleural Needle biopsy	15		21	1	37
Thoracoscopy	4		7	–	11
Thoracotomy	6	3	41	–	50
Autopsy	–		5		5

Malignant mesothelioma was diagnosed in thoracotomy in one patient although the pleural needle biopsy performed 4 times had given the result of chronic fibrous pleuritis. Another patient's pleural punch biopsy had revealed malignant mesothelioma, thoracoscopic appearance confirmed the same diagnosis but the biopsy result came as chronic fibrous pleuritis.

Skin metastases appeared at the biopsy site in two patients who had undergone thoracoscopy. In one patient pleural punch biopsy, thoracoscopic biopsies had revealed chronic fibrous pleuritis, but thoracotomy showed malignant mesothelioma. In another patient, the first thoracotomy had revealed chronic fibrous pleuritis and decortication had been performed. 4 years after his first operation he had to undergo re-thoracotomy on the same side and this time malignant mesothelioma was diagnosed.

The follow-up period of more than 2 years as well as the histological appearance played a role in the diagnosis of chronic fibrous pleuritis.

Breast cancer developed in one of the patients who were given the diagnosis of benign mesothelioma.

The tissue diagnosis was established in 75 cases. The remaining 55 cases were diagnosed clinically and radiologically. 21 of these patients were from Karain, and have died between the years of 1970 and 1974 with clinical and radiological manifestations of pleural mesothelioma.

Twenty cases of pleural malignant mesothelioma were classified from the point of view of histological types. There were 13 epithelial, 4 sarcomatous and 3 mixed form.

Table IV gives the details of 9 patients diagnosed as asbestos pleurisy. Each of the patients from Karain has at least one case of mesothelioma and/for neoplastic disease in their family history. Pathologic diagnosis was chronic fibrous pleuritis in the patients who had undergone pleural needle biopsy. Hyalinized and calcified pleural plaques typical of asbestos exposure were seen during the thoracoscopy of patient No. 6. Amphibole and chrysotile fibres together with talc, feldspar, quartz, mica and kaolinite were seen in the pleura of case 1. (Figures 6, 7).

Aflatoxin, benzpyrene and nitrosamine which are known to be oncogenic have not been found in any significant quantity in any kind of material to which the peasants from Karain are exposed or have ingested. The optic-spectrographic semi-quantitative analysis of the volcanic tuffs and soil from Karain did not show any significant quantity of carcinogenic elements such as arsenic, uranium, nickel, chronium and iron.

TABLO IV

THE DETAILS OF 9 PATIENTS DIAGNOSED AS ASBESTOS PLEURISY

Patient age, sex	Residence	Symptoms, duration	Pl. Fluid mac. appe.	Diagnostic procedures	Therapy	Follow-up period
1. O.D. 42.M.	Karain	Pain 25 years	pseudochylous	Thoracotomy	Decortication	2 years
2. M.Ö. 42. M.	"	Chest pain 6 months	–	Clinic-radiol.	–	2 years
3. O.P. 37 M.	"	Chest pain 2 years	serofibrinous	Thoracotomy	Lobectomy decortica	2 years
4. Ş.Y. 50 F.	"	Chest pain 2 years	" "	Pl. needle biopsy	–	2 years
5. M.C. 26 M.	"	Chest pain 3 months	" "	Pl. needle biopsy	–	9 months
6. S.U. 45 M	Kurşunlu Çankırı	Fever, 3 months Chest pain	" "	Thoracoscopy	–	3 years
7. M.G. 44 M.	Belçinar Tarsus	Chest pain 1 year	" "	Thoracotomy	Decortication	1 year
8. R.K. 26 M.	Çermik Diyarbakır	Chest pain 1 year	" "	Thoracotomy	Decortication	2 years
9. H.K. 65 M.	Erbaa Tokat	Chest pain 1 year	" "	Thoracotomy	Decortication	2 years

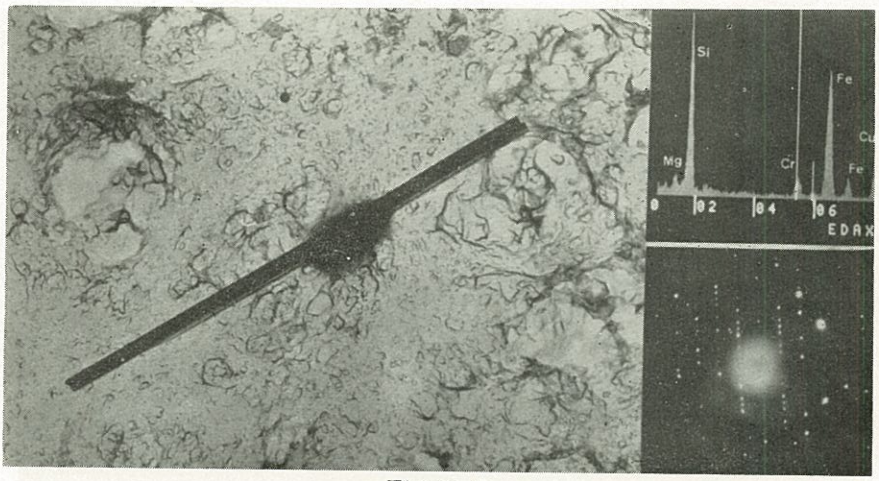

Figure 6

Amphibol asbestos fiber harvested from the pleural tissue of a patient from Karain whose diagnosis is chronic fibrous pleurisy.

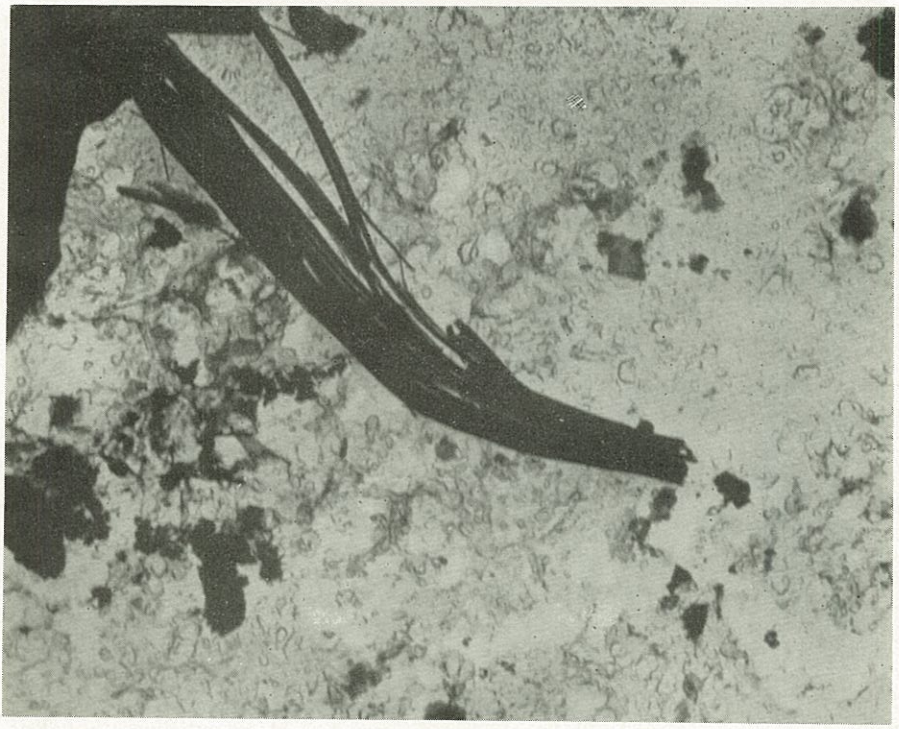

Figure 7

Chrysotile asbestos fiber harvested from the pleural tissue of the same patient.

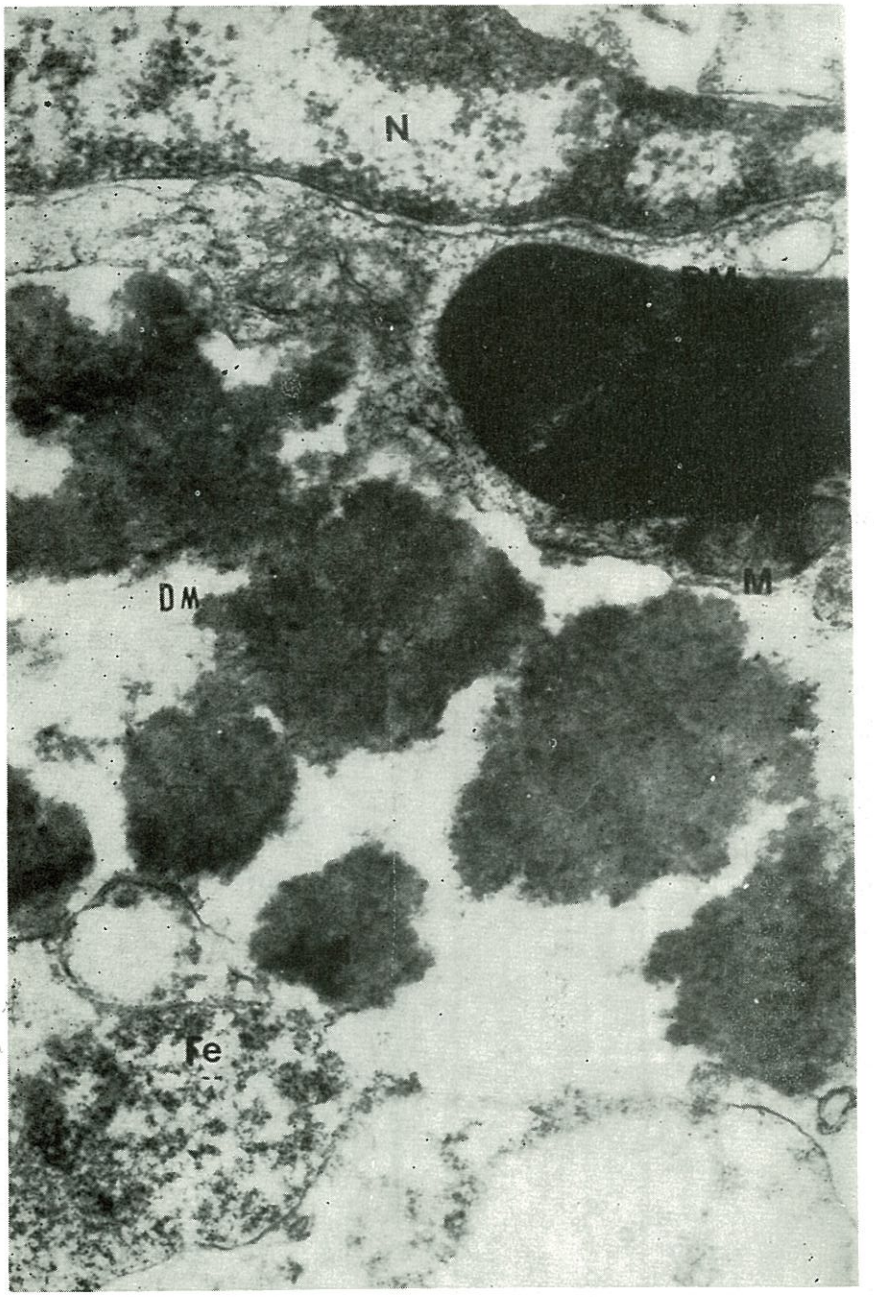

Figure 8

E.M. view of a mesothelial cell, from a case diagnosed as M. mesothelioma from Karain. N., nucleus, M.mitochondrium, DM, dense material, Fe Perritin (Lead citrate-Uranyl acetate x Jeol 100 B.78.000)

Virologic studies carried out using the 4 birds of Karain have showed that they do not carry herpes simplex viruses.

Geological studies show that there is no asbestos in the vicinity of Karain; nor any asbestos mines, mills or factories are located there. Optical and E.M. examination of the volcanic tuffs, stucco and various samples of the soil to which the villagers were exposed revealed mainly colourless, irregular, striated volcanic particles from 50 millimicrons to submicrons and many fibres from 75 millimicrons to submicrons. These were not like asbestos. The mineralogical examination of the dust to which Karainers were exposed showed clay minerals, calcite, feldspar, volcanic glass, quartz, biotite, chlorite, muscovite and augite. But the geological and mineralogical examination of the soil from the roads, fields, and white stucco obtained from Erbaa/Tokat, Mihalliççik/Eskişehir, Maden/Elazığ and Çernik/Diyarbakır showed tremolite asbestos fibres along with considerable amounts of other silicates such as mica, talc, feldspar and kaolin.

Electron microscopic examination of the pleural pieces of 5 patients showed dense material inside the mesothelial cells (Figure 8). The same condition was present both in the patients with malignant mesothelioma and asbestos pleurisy.

Asbestos fibres in Karain's tap water were investigated. Yet non-identified asbestiform fibres were seen during the analysis (Figure 9).

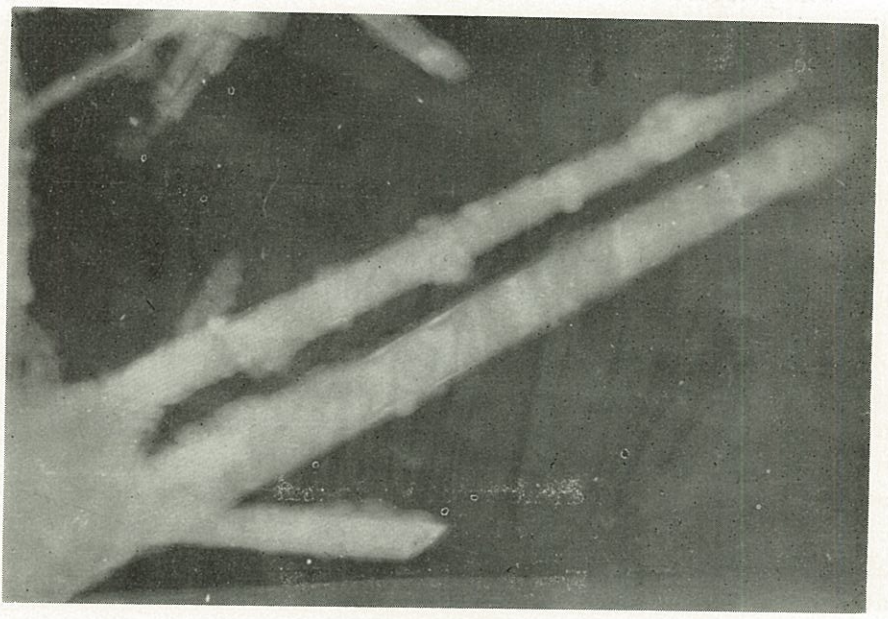

Figure 9

Asbestiform fibers in the tap water of Karain.

Follow-up of the 46 malignant pleural mesothelioma cases showed 34 rapid and 12 slow progression.

Therapeutic measures: Among the 41 pleural malignant mesothelioma cases who had undergone thoracotomy, 5 patients had decortication or pleurectomy, 1 pleurectomy plus thoracoplasty, 2 pneumonectomy. Fibrous benign mesothelioma in three cases were excised.

Chemotherapy: 7 patients had platinium IV, 3 nitrogen mustard IV., 2 adriamycin, 2 methyl CCNU , 1 oral endoxan, 1 intrapleural nitrogen mustard and 1 intrapleural thiotepa. One patient had 2 doses of adriamycin along with intrapleural nitrogen mustard, another had intrapleural nitrogen mustard plus per oral endoxan. Only malignant mesothelioma cases had chemotherapy.

Discussion

The average age of our patients is the same as in the literature. Young patients as some of our cases have also been reported.[34] The sex ratio of the patients seems to be interesting. The disease affects both sexes equally, which shows that it is not occupational.

The pedigrees of the Karain villagers are very interesting. The disease is familial but not due to a genetic defect. The fact that other types of cancer are also seen within the family, suggests that the cause may be asbestos. Such cases have been reported in the English literature.[35, 36]

The clinical manifestations of our patients are not different from those reported. Hence, rare findings such as vena cava superior obstruction and Horner's syndrome have been encountered in two of our cases.[37, 39] The radiological and laboratory findings, differential diagnosis and the prognosis are not different from published cases.

Among the 120 cases examined, only 2 were exposed to asbestos occupationally. The remaining 118 patients all happen to be peasants, living in a rural area. However 16 patients from Tokat, Elazığ, Eskişehir and Diyarbakır regions are found to be exposed to asbestos types of actinolite and tremolite environmentally. In some of the mesothelioma cases reported it can be seen that there was no known exposure to asbestos in many patients.[35-37, 40-45] It is difficult to accept these cases as unnoticed. This fact is made clear with careful questioning; it is a remote possibility, not to be exposed to such a substance like asbestos, as it is used in more than 1000 kinds of occupational working places.

Is it possible to relate the mesothelioma and asbestos pleurisy directly to actinolite and tremolite asbestos when we accept for a moment that these peasants have inhaled such minerals environmentally? The answer to this question cannot be given easily, because the conclusions reached by the advisory committee on asbestos cancers at the Lyon meeting, point out that "although all commercial fibers except anthophyllite, but including talc have been implicated, there are important between-fiber differences in mesothelioma risk being greater with crocidolite, less with amosite, and apparently even less with chrysotile. With amosite and chrysotile, there appears to be a higher risk in manufacturing than in mining and milling".[4]

The Anatolian peasants use the "white soil", which usually has clay, kaolin, mica, calcite and talc in it and sometimes asbestos; as an ingredient in producing "pekmez", wine, grape juice, as stucco material and in the isolation of roofs, also as tooth paste and toilet substance for their babies. This soil is used in some regions in making pottery. It is almost impossible to find an Anatolian peasant who is not exposed to this substance. It is difficult to relate the endemic pleural calcifications in Anatolia directly to asbestos probably present in this white soil, as we have not been able to find asbestos in the soil specimens which come from villages with endemic pleural calcifications.[28] Similarly, there is an increase in the prevalence of pleural calcifications in some agricultural populations in which the soil contains asbestiform minerals.[47, 48] Rous and Studeny,[49] in their study of the endemic pleural plaques in the region of Pelhrimov of Czechoslovakia have reported that there was no asbestos in the environment. When there is no occupational and indirect asbestos exposure, but asbestos fibers are detected in the pleural tissue of the persons with calcified pleural plaques, with mesothelioma and asbestos pleurisy, three possibilities are considered: (1) The above mentioned diseases are related to silicate minerals other than asbestos, such as talc, mica, kaolin and feldspar. (2) Tap water, beverages and the food are the sources of asbestos fibers. (3) These three diseases have other causes such as arboviruses which are indicated among the causes of mesothelioma.[50] Benzpyrene, aflatoxin, nitrosamine and redioactive elements were not found in the soil of Karain.

Attention has recently been directed toward the widespread occurrence of asbestos fibers in certain natural sources and beverages.[51-54] There is no agreement about the fact that the asbestos fibers in the water may be hazardous to health. Animal studies carried out by Gross et al have shown that the penetration of the ingested asbestos fibers through the walls of the G-I tract does not occur.[55] Gross[56] reports that

the asbestos fibers in the water and beverages are submicronic (short-fibered (< 5 millimicrons in length) and the asbestos fibres of this kind are not carcinogenic and fibrogenic. However some authors have suggested that the direct inoculation of asbestos fibres to the stomach results in penetration of the G-I tract by these fibres and spreading to various organs, and this condition may be important in asbestos carcinogenesis.[57-59]

Although the report of the advisory committee emanating from the Lyon Conference concluded that there is no evidence at present of "an increased cancer risk resulting from asbestos fibres present in water, beverages or food or in the fluids used for the administration of drugs", the outbreak of mesothelioma in Karain may well be an example of this condition. The presence of this disease in only one of the three villages located at a distance of 3-4 kilometers from each ofter and in the same valley, where the same kind of inhabitants live in the same living conditions, is difficult to explain. The only difference in the living conditions in these villages is the source of the water used. This difference supports the probability that the disease is caused by asbestos fibres, from water.

It is shown that natural origins of asbestos are found in the water of rivers derived from the wearing down of asbestos-containing rocks. We are planning to carry out studies on the water of Karain.

Clinical and epidemiological studies have shown that occupational and indirect exposure to asbestos may lead to pleural mesothelioma in the population,[8, 35, 36, 42, 45, 60-63] and asbestos pleurisy.[35, 64-72] Two factors have always drawn attention to this subject. The first of these is the relationship between pleural mesothelioma and asbestos pleurisy. It has been reported that some of the asbestos pleurisies, accepted as benign, may turn to malignant mesothelioma.[64, 67] Some of the patients who have had a diagnosis of malignant mesothelioma have a pleural effusion in their past histories.[60] Secondly, it is shown that diagnosing asbestos pleurisy and malignant pleural mesothelioma may sometimes be very difficult and these two diseases may lead to confusion.[68, 73, 74] These factors have also been observed in some of our patients. The disagreement on the diagnostic methods, the fact that no important difference can be shown between asbestos pleurisy and malignant mesothelioma, even by electron microscopic examination, and that a patient developed mesothelioma long after he had undergone decortication, because of chronic fibrous pleuritis, confirm this confliction. Therefore we quite agree with Becklake's statement on this subject: "One could argue

against accepting the diagnosis of benign asbestos pleural effusion until all of the present reported cases are followed to death, and death is shown to be attributable neither to carcinoma of the lung nor to mesothelioma."[75] In establishing the diagnosis of asbestos pleural effusion, it is very important to show asbestos fibres in the tissue by minerological examination along with the clinical, radiological, and pathological manifestations. We believe that, Geansler is right in his consideration that a great majority of the cases accepted as idiopathic pleurisy may in fact be asbestos pleurisy.

It is shown that mesotheliomas may develop from the pleural calcifications accepted as benign, by some authors.[76-79] Pleural calcification was seen together with malignant mesothelioma, in one of our Karain patients. In another patients biopsy material, mesothelioma together with calcification was seen. In yet another patient with benign pleural mesothelioma, scattered pleural calcifications were noticed during thoracotomy. We think that both pleural plaques and asbestos pleurisies must be accepted as the precursors of pleural mesothelioma and these patients should carefully be followed.

In the absence of a controlled trial, it is not possible to determine whether the surgical and medical treatment with cytotoxic agents used in the cases of our malignant pleural mesothelioma series are effective or not. We agree with Elmes'[39] point of view on this subject.

Summary

In this study, 120 cases of pleural disease caused by asbestos are presented. 108 of these cases were malignant pleural mesothelioma, 9 asbestos pleurisy and 3 benign pleural mesothelioma.

In the 120 total cases, only two cases were occupational asbestos exposure, the other 118 included peasants living in rural areas, 37 of whom resided in the village of Karain. Sixteen patients with a history of environmental asbestos exposure come from provinces of Tokat, Eskişehir, Diyarbakır and Elazığ. No condition that may result in the inhalation of asbestosic material was encountered in the rest of the cases. In such cases it is suggested that the asbestos causing asbestos pleurisy and pleural mesothelioma, may have come from water, beverages or food, or from other sources.

The relationship between malignant pleural mesothelioma and asbestos pleurisy is discussed and difficulties in diagnosis are emphasized. The significance of showing asbestos fibres in the tissue by mineralogic, E.M. examination is stressed along with the clinical, radiological and pathological appearance in the diagnosis of these two diseases.

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A Giant Typhoid Liver Abscess Responsive to Medical Treatment

(A Case Report)

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Cholecystitis and liver abscess are well-known complications of typhoid fever. Usually the abscesses are small and require no surgical treatment. This is a report of a case with a giant liver abscess which responded to medical treatment.

Case Report

A 46 year-old white male was admitted to the Hacettepe University Medical Center for the first time with the complaint of abdominal pain. Three weeks prior to admission he had developed a fever which elevated up to 39°C. Several days later he started to have generalized abdominal pain and was placed on broad spectrum antibiotics; however, he did not respond to this treatment. Abdominal pain increased and was localized in the right upper quadrant and he was admitted to the hospital for investigation.

Upon admission, physical examination revealed an acutely ill, well-developed male with the following findings: Blood pressure, 120/70 mm Hg, Pulse, 68/min, temperature, 38°C. There was dullness and diminished respiratory sounds in the right lung base. The liver was tender and enlarged, 6 cm below the right costal margin. The spleen was enlarged, 1 cm below the left costal margin.

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Laboratory Findings: Hbg: 9.85 gm. %, Hematocrit: 37 %, WBC: 3000 cu mm, Urinanalysis: normal, BUN: 10 mg %, Blood sugar: 86 mg %, Serum bilirubin: 0.7 mg %, Alkaline phosphatase: 19 K.A. units, SGOT: 75 units, SGPT: 56 units, Serum protein: 7.5 gm %, Alb: 2.7 gm %, Glob: 4.8 gm %, Serum Amylase: 40 S.U., Cholesterol: 234 mg %, Total lipid: 940 mg %, Casoni-Weinberg Test: negative. Brucella Agglutination: negative, Salmonella Typhi agglutination: H. 1/400; 0. 1/200 positive; Chest X-ray: lung clear, right diaphram was elevated, Liver Scan: performed with ^{99m}Tc-S-Colloid showed a round space-occupying lesion 15 cm in diameter, suggesting a cyst or abscess (Figures 1 and 2).

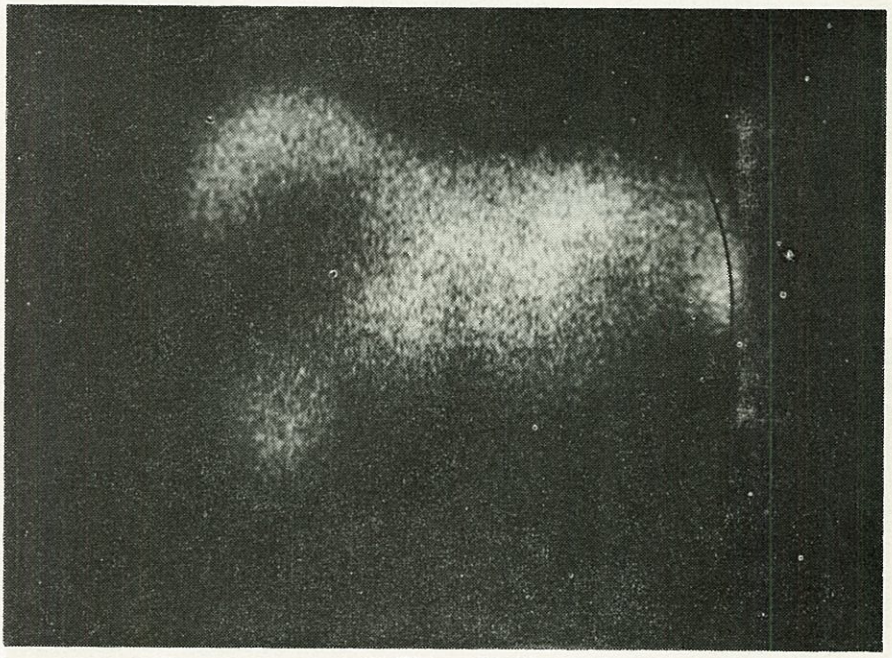

Figure 1
Anterior view of the liver scan.

Course in the Hospital: A diagnosis of typhoid fever and possible liver abscess or infected hydatid cyst was made. The patient was placed on Ampicillin at dosages of 4 gm/day and Gentamycin of 120 mg daily. One week later his temperature dropped to normal, his general condition improved and liver tenderness decreased. The patient continued antibiotic treatment and 3 weeks later liver scanning was repeated and showed some improvement (Figures 3 and 4). The patient refused to stay in the hospital and was discharged in good condition.

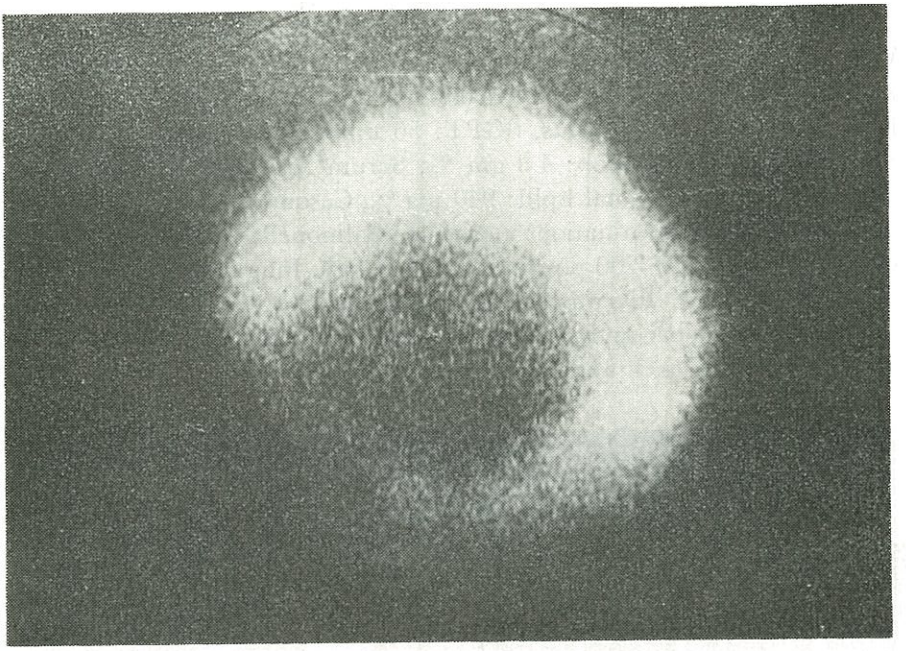

Figure 2
Lateral view.

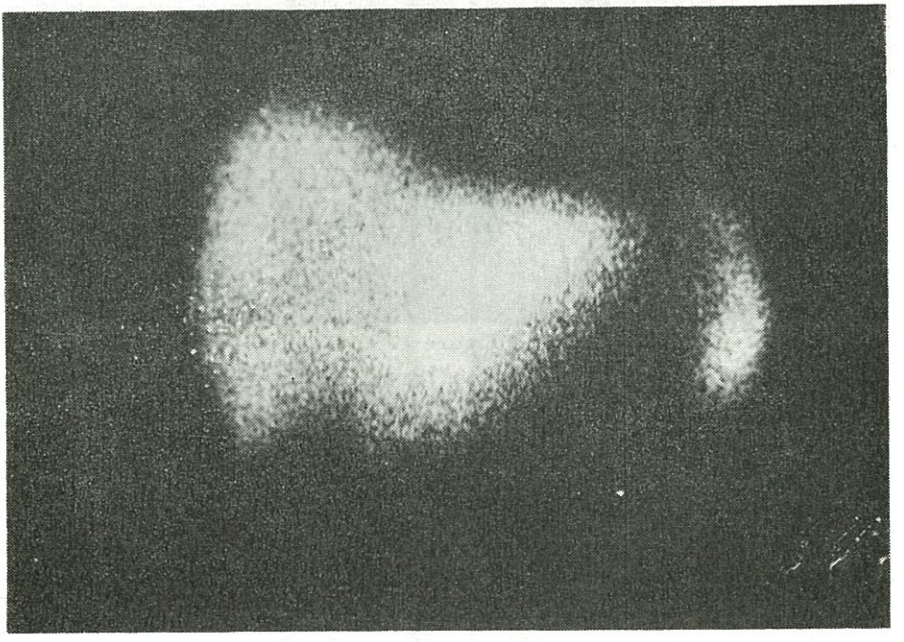

Figure 3
Anterior view.

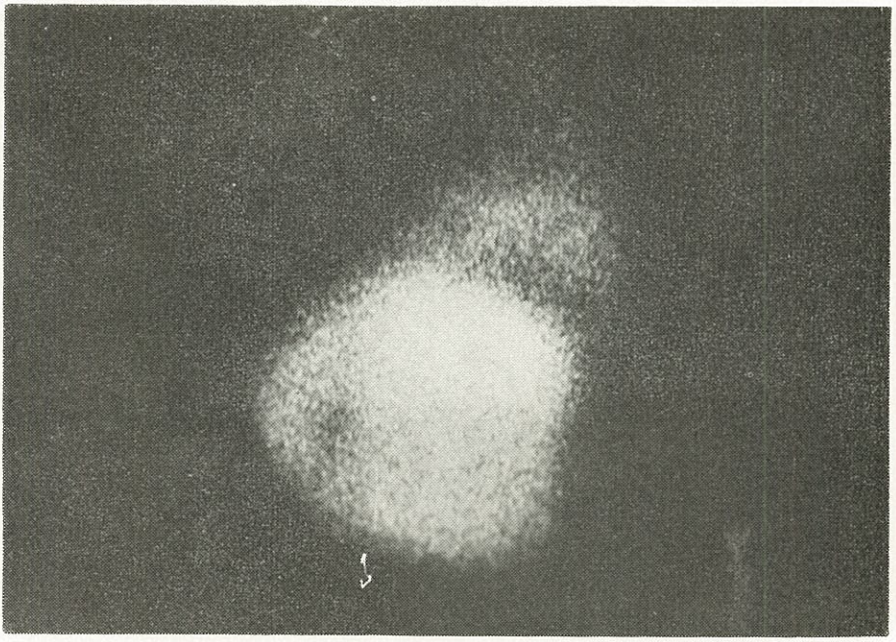

Figure 4
Lateral view.

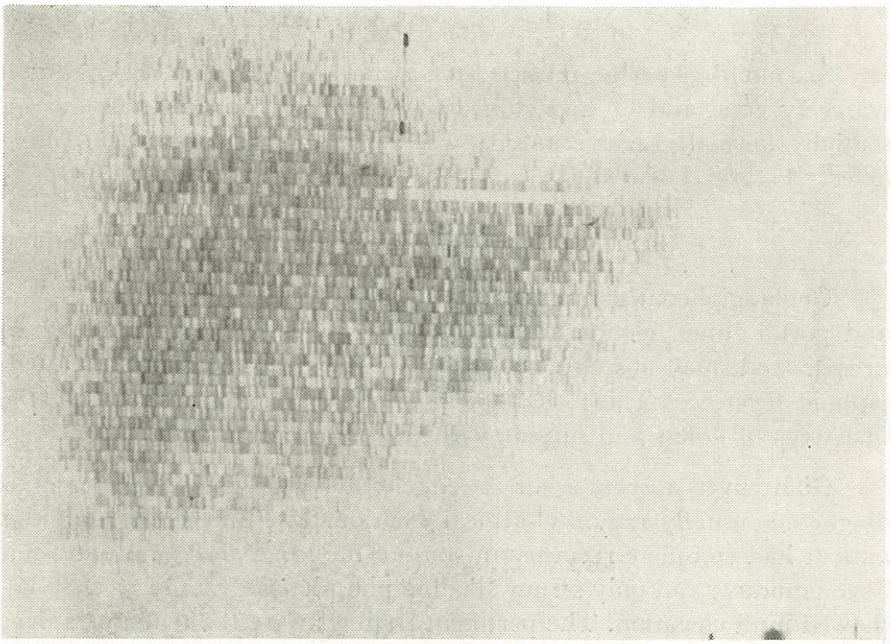

Figure 5
Anterior view.

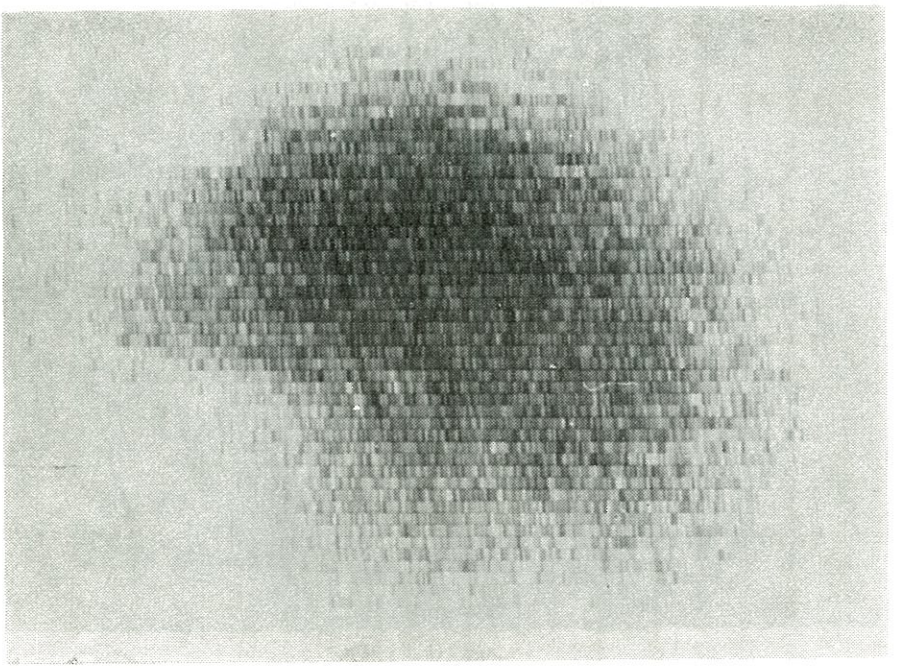

Figure 6
Lateral view.

Six months later he returned for a check-up. His general condition was very good and he had gained 6 kg. Physical examination was essentially normal. Liver scanning was repeated and showed no filling defect (Figures 5 and 6).

Comment

Cholangiohepatitis, cellular infiltration of the liver paranchyma and portal zones, cloudy swelling, proliferation of Kupffer cells, focal necrosis and small abscesses are fairly common findings in patients with typhoid fever.[1, 2, 3] These changes in the liver usually occur after the first week of illness and subside with the patient's recovery.

Giant liver abscess is an infrequent development and when it is diagnosed, usually requires surgical excision. It is interesting that our patient had complete recovery in spite of the large abscess. He did not have jaundice, and only serum alkaline phosphatase, SGOT and SGPT showed mild elevation. The pertinent findings were the tenderness over the liver, an elevation of the right diaphragm and an abnormal liver scan.

Summary

A case of giant typhoid liver abscess is reported. The patient responded well to medical treatment and required no surgical excision.

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An Electron Microscope Study on the Prenatal Development of Mouse Lung

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Classically, the intrauterin growth of the human lung is divided into three periods:[1, 2, 3, 4, 5]

Glandular Period: This occupies a period of up to approximately 16 weeks of gestation. During this time the endodermal bronchial tree ramifies through mesenchymal tissue.

Canalicular Period: In this period (up to 24 weeks), there is a more rapid growth of the mesenchymal tissue associated with the peripheral regions of the bronchial tree.

Alveoler Period: This period of development occupies the period from 24 weeks to full term. The lung loses its glandular character and becomes increasingly vascular. The bulbous expansions at the ends of the bronchial tree branch further, and alveoli arise as shallow evaginations from the side of the channel's walls.

Most of ultrastructural studies on the development of animal lungs have usually been carried out on the late embryons and investigated in three periods as considered in human lungs.[6, 7, 8, 9] Findings obtained from these studies, did not give detailed information about the cell differentiation, taking place in the endodermal epithelial cells of the bronchial tree and in the surrounding mesenchyme of the lung tissue during the development.

In this study, starting from the earliest mouse embryon up to full term, differentiations of the endodermal and mesenchymal cells were

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investigated at daily intervals during the genesis of the lung at the electron microscopical level.

Material and Method

Female Swiss albino mice in estrus were mated overnight. The day following a successful mating, assessed by the presence of a vaginal plug, was considered the 1st day of gestation (normal gestation time is 21 days). From day 12 through 20-21 day of gestation, pregnant mice were anesthetized with ether and the fetuses were removed from the uterine horns through an anterior abdominal incision.

The embryons containing lungs and the embryonic lung tissue slices were first fixed in a solution of 2.5 % glutaraldehyde and then in a solution of 1 % osmium tetroxyde. After dehydrating at room temperature the tissue slices were embedded in araldite (araldite CY 212, Taab). The thin sections were obtained from blocks of araldite, using Porter-Blum MTI ultramicrotome and glass knives. Sections were collected on unsupported grids and stained with phosphotungstic acid (PTA) in acetone for 1-2 minutes and later on with 70 % uranyl acetate saturated in ethanole for 30 minutes.

All the sections were studied under the electron microscope, Carl Zeiss EM 9 A. The photographs were taken using Agfa-Gevaert Sciencia films and prints were made on Kodak bromide and Ilford paper.

Observation

12- day-old embryons: The lungs of these embryons consisted of primitive bronchi and a vascular mesenchymes. The endodermal epithelial cells, lining primitive bronchi were stratified epitheliums and separated by large intercellular spaces. These cells were based on basal laminae. At the luminal border they were attached to each other by tight junctions. Their apical surfaces showed microvilli (Figure 1). According to their cytoplasmic organelles they were identified as two types; dark cells and light cells.

Dark cells were scanty among the white cells (Figure 1). They contained abundant ribosomes scattered homogenously, a few mitochondria and a small amount of granular endoplasmic reticulum. Their nuclei were large, irregular and heterochromatic (Figure 2).

Light cells were rich in ribosomes which accumulated in different areas of cytoplasms. Their granular endoplasmic reticulum was little and mitochondria were few in number. They had large, ovoid and euchromatic nuclei and well-developed nucleoli (Figure 2).

16- day-old embryons: Their lungs were found to be glandular in appearance. Solid bronchiolar buds and canalized bronchioles branched through the mesenchymes. Bronchioles were lined by prismatic endodermal epitheliums (Figures 3 and 4). The intercellular spaces between epithelial cells became narrowed. They rested on basal laminae and had numerous microvilli and tight junctious at their apical aspects (Figure 5).

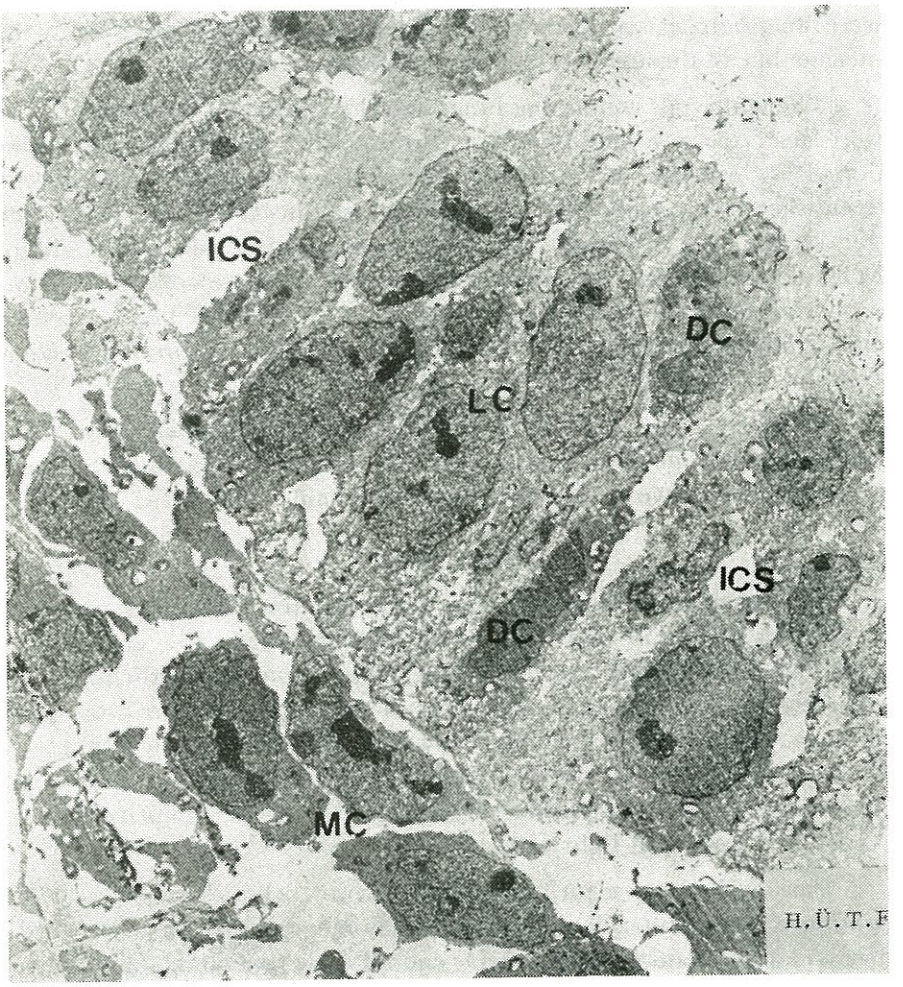

Figure 1

Dark (DC) and light endodermal cells (LC) among the stratified columnar endodermal epithelial cells, lining the primitive bronchus in the 12-day-old mouse embryon are seen. Intercellular spaces (IS) between endodermal cells and mesenchymal cells (MC) around the primitive bronchus attract attention (X 6600).

The identification of the dark endodermal epithelial cells was more easy than those of 12-day old embryons. They were also observed rarely among the light endodermal cells (Figures 3, 4 and 5). Their cytoplasms had a few mitochondria, a small amount of granular endoplasmic reticulum and abundant ribosomes and glycogen, often concentrated in different areas of cytoplasms (Figure 6).

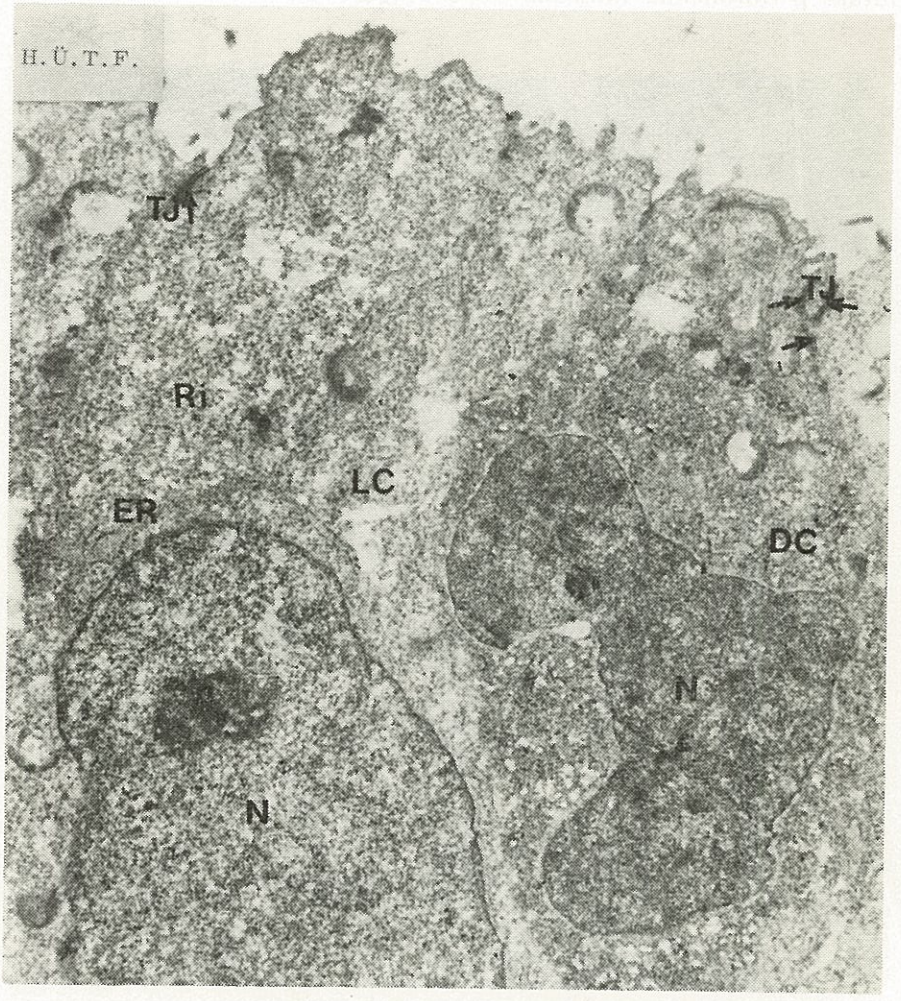

Figure 2

n 12-day-old embryon dark cell (DC) and light cell (LC) are attached to each otherf by tight junction (TJ) at their luminal border and their apical surfaces show microvilli (Mv). Differences between two endodermal cells from cytoplasmic organelles point-o view are observed (X 18000).

The light cell, forming most of endodermal epithelial cells were rich in scattered ribosomes and glycogen, and contained well-developed granular endoplasmic reticulum, Golgi complexes and a few round mitochondria (Figure 5).

Some undifferentiated mesenchymal cells accumulating around the bronchioles were differentiated into smooth muscle cells in order to form future peribronchial muscular coats (Figure 4).

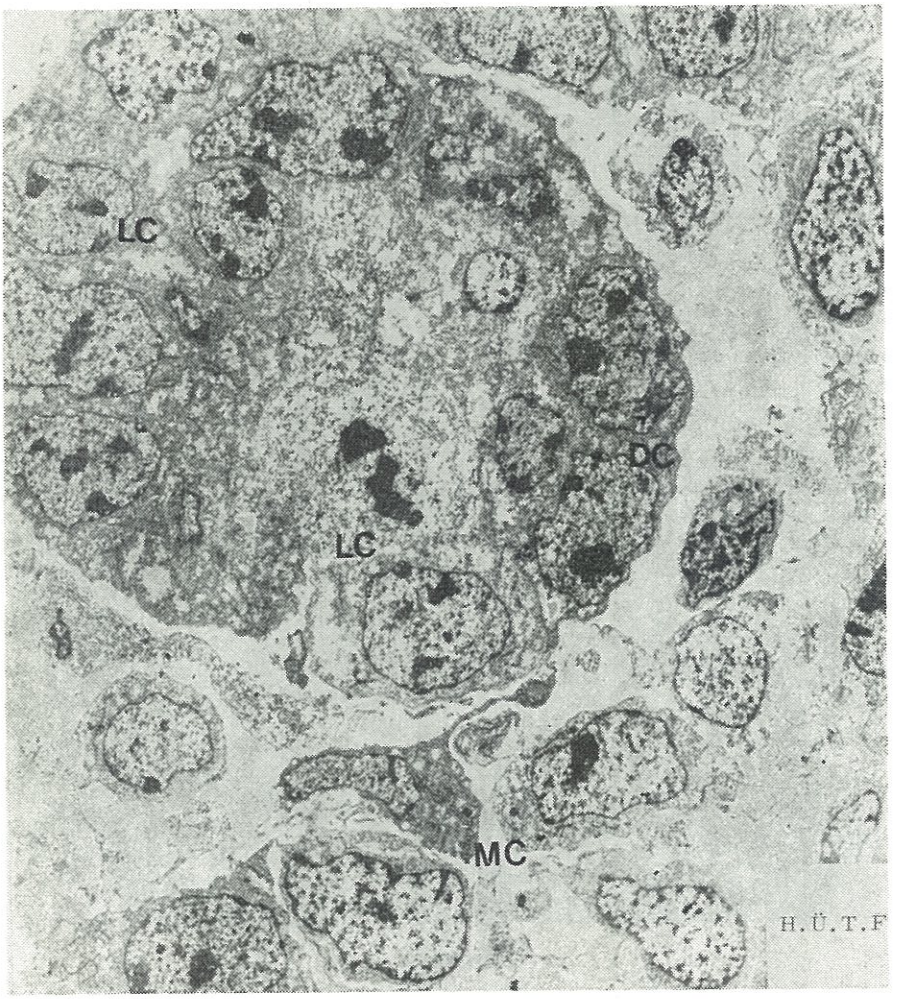

Figure 3

A solid bronchiolar bud having dark (DC) and light cells (LC), and surrounding mesenchymal cells (MC) in the 16-day-old embryon (X 6600).

17- day-old embryons: Solid bronchiolar buds were rarely seen in the vascular mesenchymes (Figure 7). Canalized bronchioles were lined by columnar endodermal epitheliums. They had basal laminae. Their apical surfaces were rich in microvilli. The tight junctions, attachhed to the endodermal epithelial cells were also observed predominantly (Figures 8, 9, and 10).

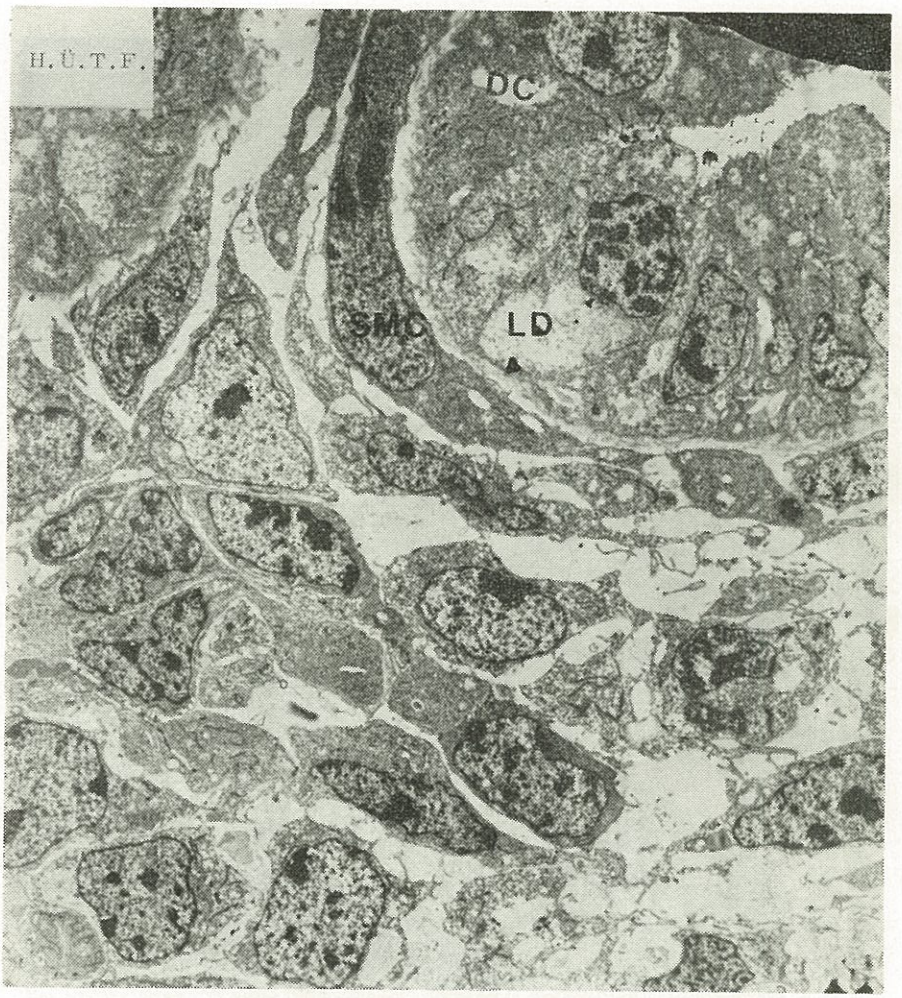

Figure 4

Canalized bronchiole having dark (DC) and light cells (LC), smooth muscle cell (SMC) differentiated from mesenchymal cells, and surrounding mesenchyme are seen in the lung of the 16-day-old embryon (X 6600).

Dark cells attracted attention among the light cells. They were few in number as observed in the early embryons (Figure 8). Their columnar shapes tapered towards the lumina (Figures 8 and 9). A few round mitochondria, little granular endoplasmic reticulum, rare lipid droplets, abundant ribosomes and glycogen, accumulating in one area of the cytoplasms were found in these cells. They had irregular and heterochromatic nuclei (Figure 9).

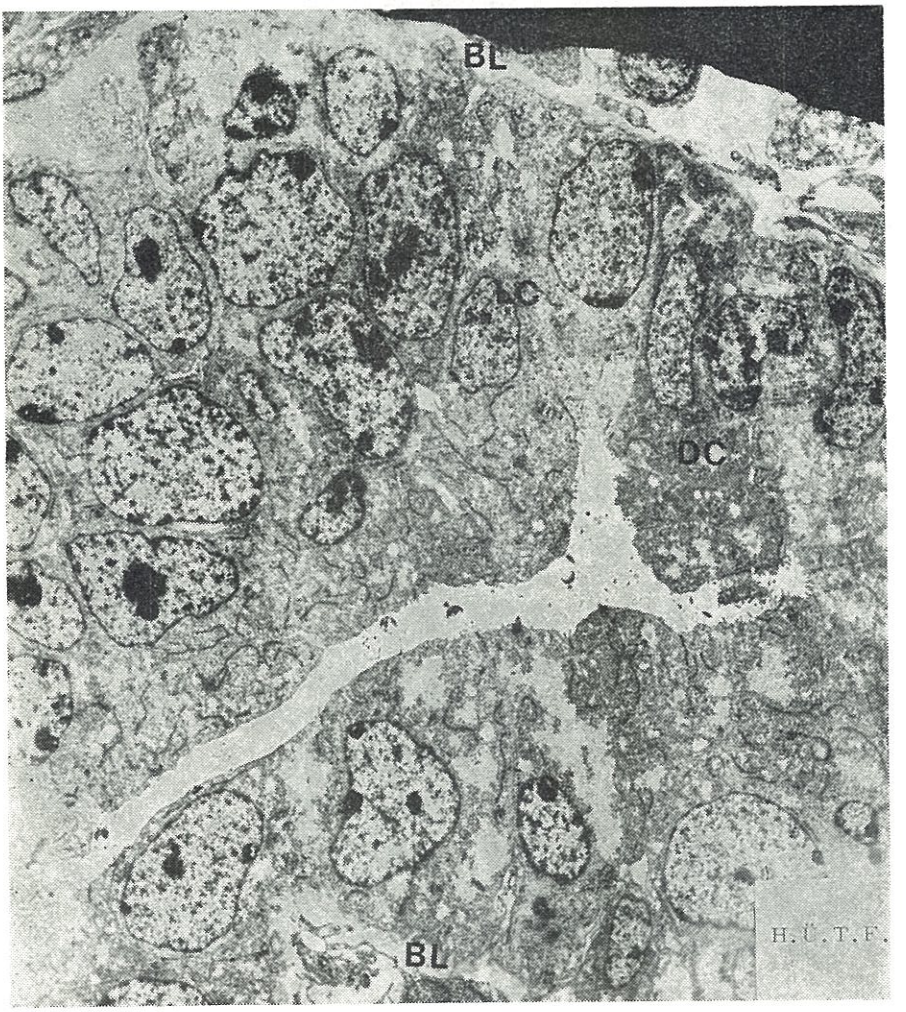

Figure 5

Dark (DC) and light cells (LC) and their basal lamina in the bronchiole of the 16-day-old embryon (X 6600).

In light cells, granular endoplasmic reticulum and Golgi complexes were well developed. In addition to these they had abundant ribosom and glycogen, and a few mitochondria inclusion bodies and lipid droplets (Figure 10).

The capillaries in the surrounding mesenchymes started to appear in the vicinity of the bronchioles. Some of them showed a close relationship with the endodermal epithelial cells (Figure 9).

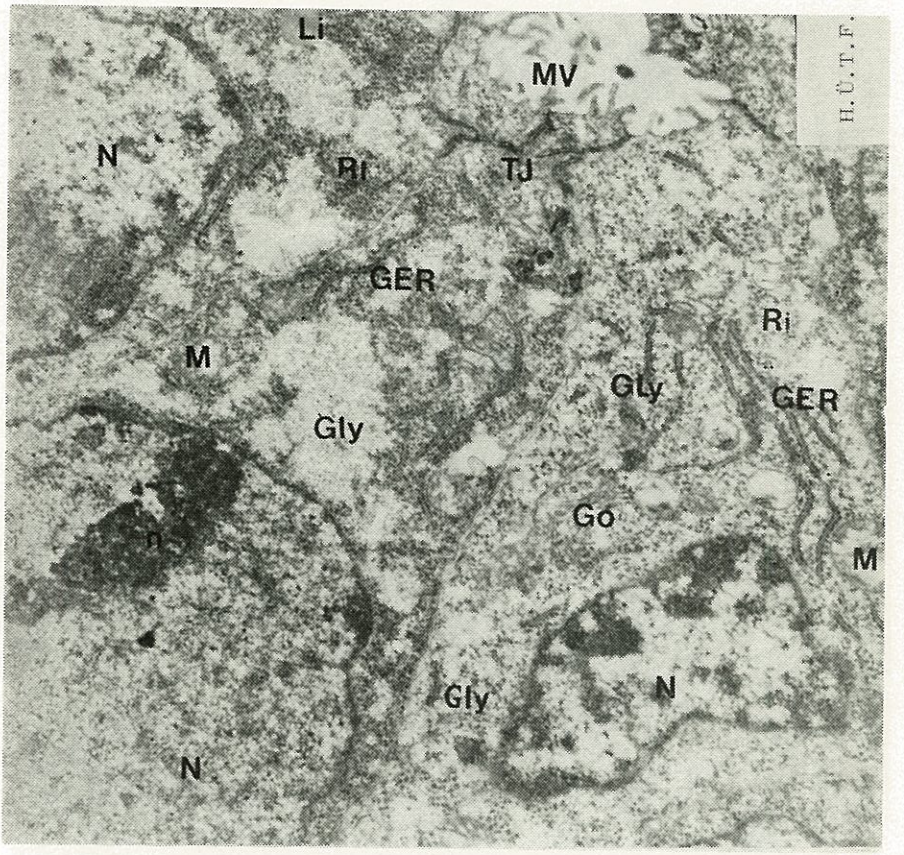

Figure 6

Dark cell (DC) having a few mitochondria (M) and lipid droplets (Li), a small amount of granular endoplasmic reticulum (GER), abundant ribosomes (Ri) and glycogen (Gly), often concentrated in different areas of cytoplasm, and light cell (LC) composed of scattered ribosomes (Ri), glycogen (Gly), well developed granular endoplasmic reticulum (GER) and Golgi complex (Go) and a few mitochondria (M) are observed in the 16-day-old embryon. Their apical aspects show microvilli (Mv) and tight junction (TJ). (X 18000).

18- day-old embryons: The lungs of these embryons had a canalicular appearance. Many small and large primitive respiratory bronchioles were seen throughout the vascular mesenchymes. They were lined by cubic endodermal epitheliums. Prominent aggregates of glycogen, many ribosomes, a few mitochondria and a small amount of granular endoplasmic reticulum were observed in their cytoplasms. Their nuclei were round and

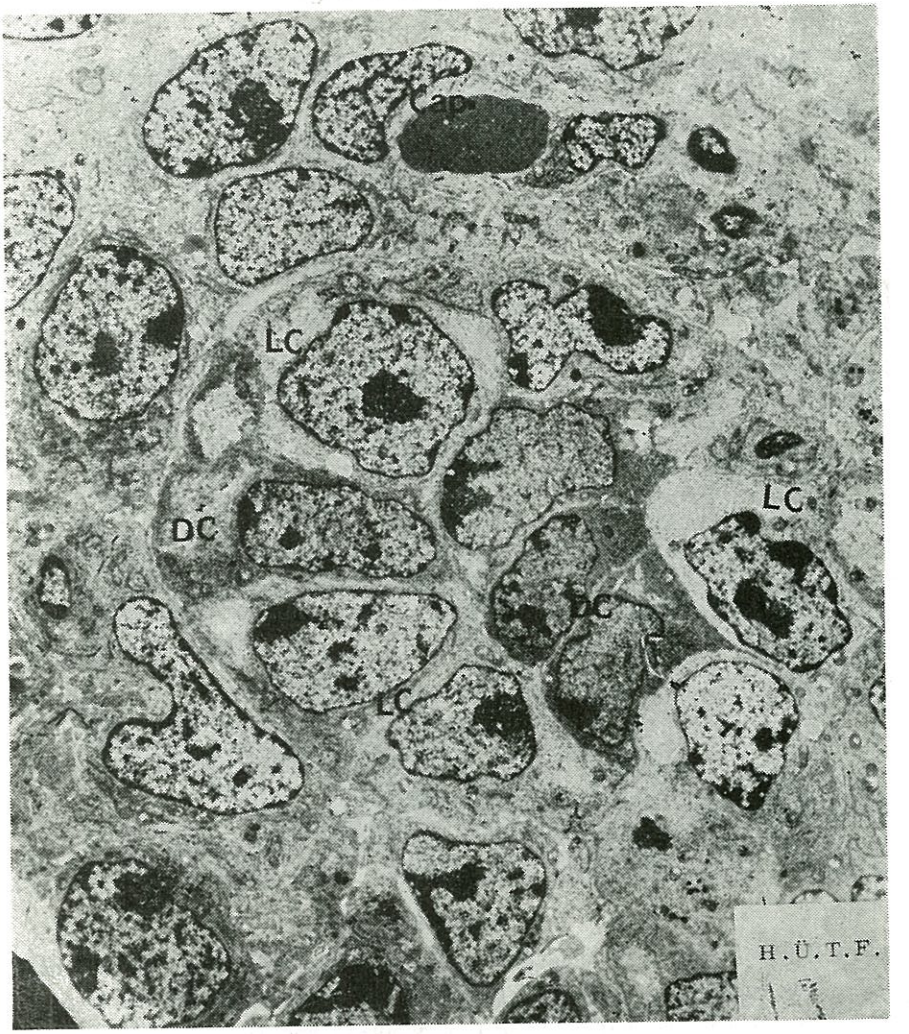

Figure 7

In the 17-day-old embryon, a solid bronchiolar bud is observed. Dark cell (DC) and light cell (LC) are identified more easily than early embryons. Capillary (Cap) in surrounding mesencyme attract attention (X 6600).

well-developed and had more than one nucleoli. The cubic endodermal epithelial cells rested on basal laminae (Figure 11).

Vascular mesenchymes displayed wide septations because of the primitive respiratory bronchioles. In these septums many capillaries established direct contact with the lining cuboidal endodermal cells.

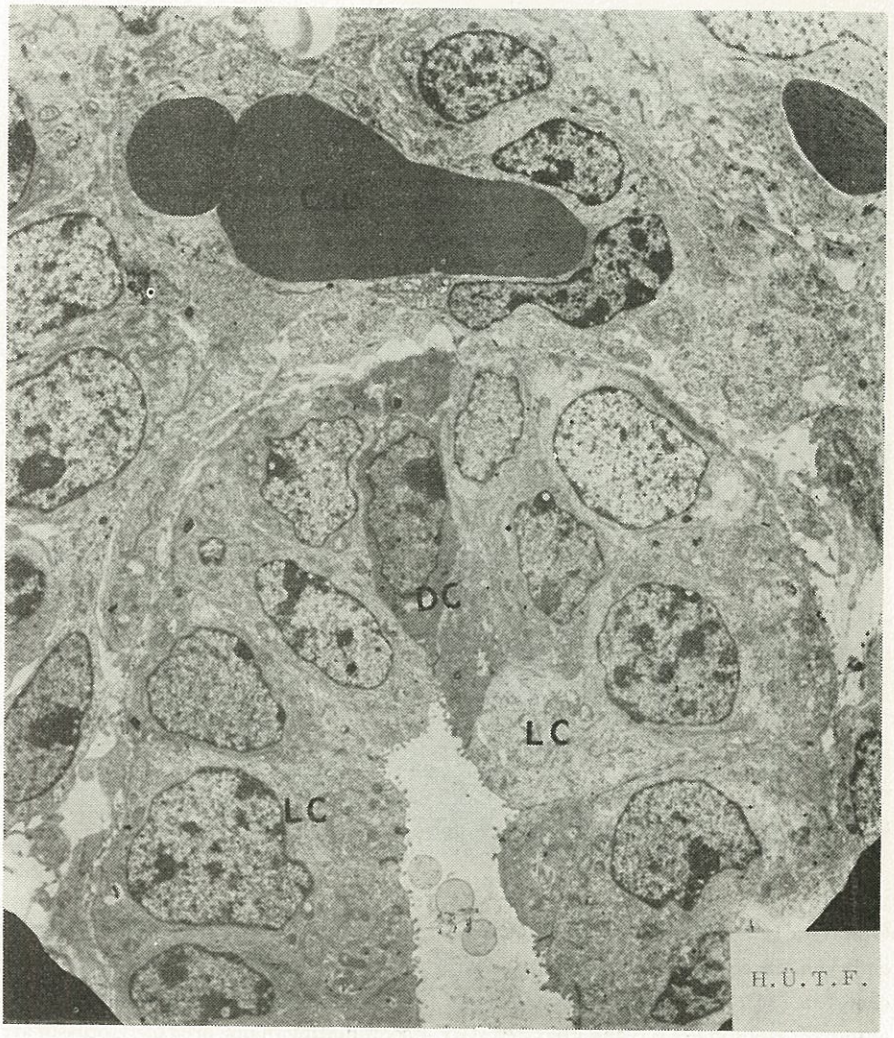

Figure 8

A bronchiole lined by columnar endodermal cells is seen in the 17-day-old embryon. Capillaries in surrounding mesenchyme appeared in the vicinity of the bronchiole (X 6600).

Some undifferentiated mesenchymal cells were differentiated into the fibroblasts which started to release the intercellular fibrillar elements (Figure 11).

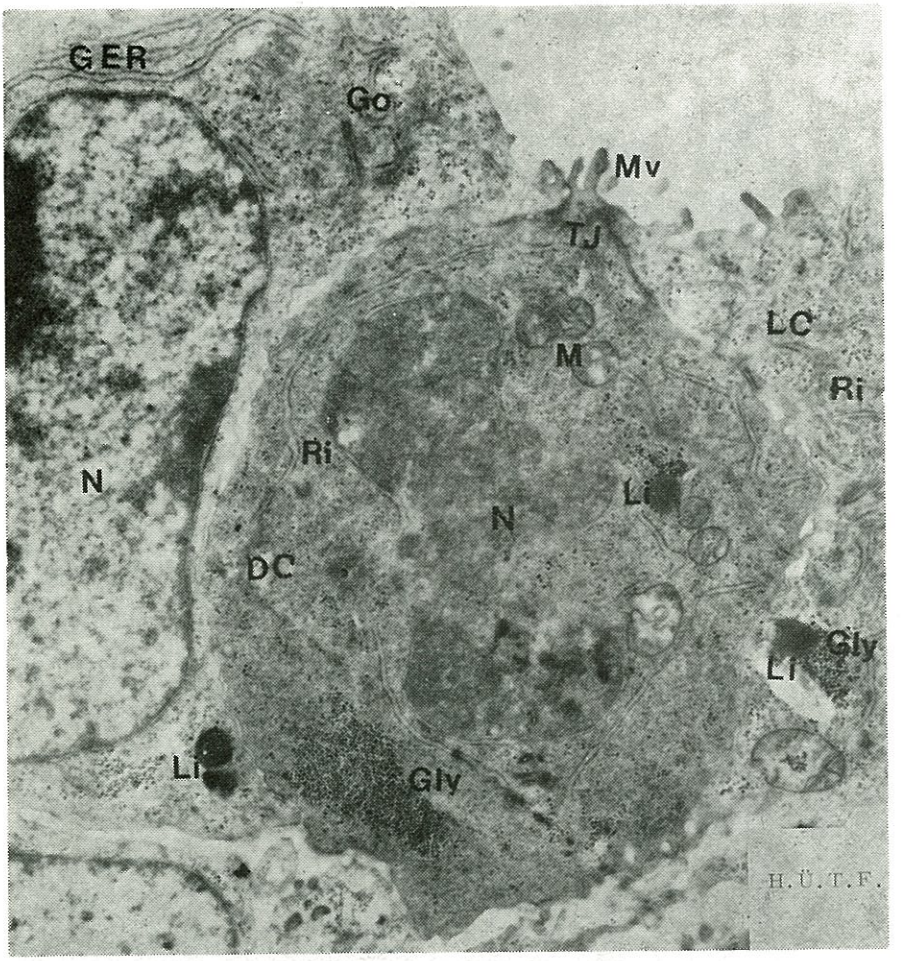

Figure 9

One dark cell (DC) between two light cels (LC) attract attention in the 17-day-old embryon. Its columnar shape tapers towards the bronchial lumen. A few round mitochondria (M), little granular endoplasmic reticulum (GER), rare lipid droplets (Li), abundant ribosomes (Ri) and glycogen (Gly) accumulating in one area of cytoplasm are found in this cell. It has irregular and heterochromatic nucleus (N). Microvilli (Mv) and tight junction (TJ) are noticed at the luminal surface. Light cells (LC) contain, scattered ribosomes (Ri) and glycogen (Gly), rare lipid droplets (Li) and well-developed granular endoplasmic reticulum (GER) and Golgi complexes (Go) (X 18000).

19- day-old embryons: Lungs displayed alveolar periods. Alveoli appeared as shallow evaginations from the side of the primitive respiratory bronchioles, walls. The most prominent findings in these lungs were observed to be the inclusion bodies in Type II cells, the blood-air barrier and Type I cells. With all these morphological developments, the terminal branches of bronchial tree were similar to the primitive alveoli (Figures 12 and 13).

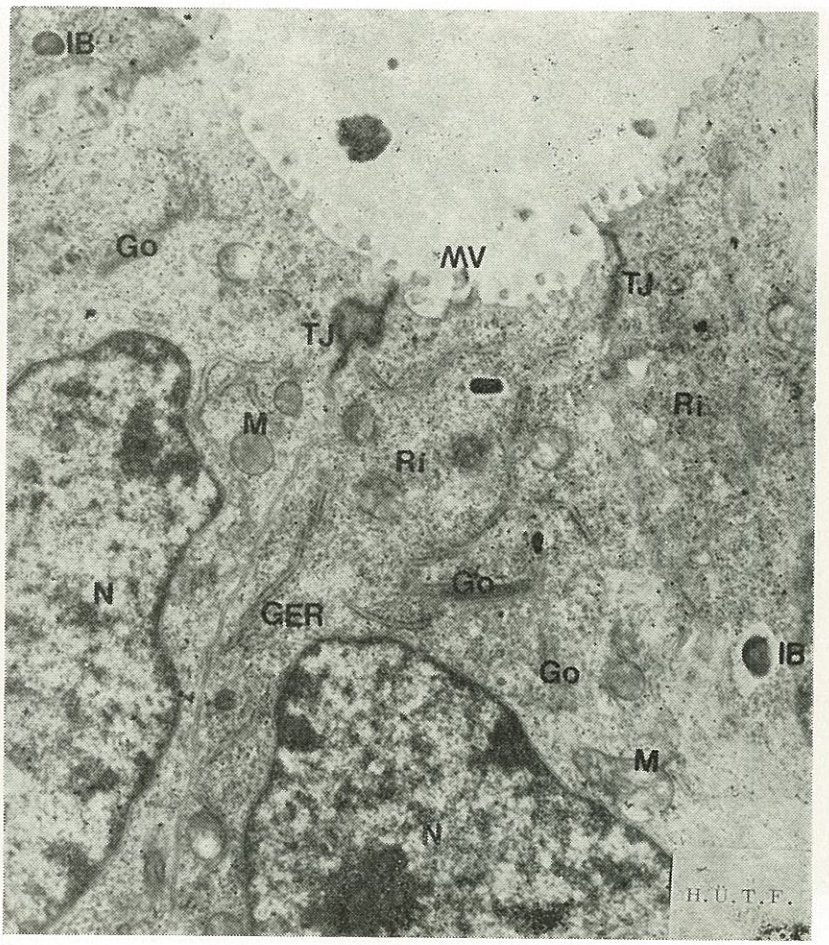

Figure 10

In the 17-day-old embryon, light cells (LC) have abundant ribosomes (Ri), a few mitochondria (M), and inclusion bodies (IB). Their granular endoplasmic reticulum (GER) and Golgi Complexes (Go) are well-developed. Microvilli (Mv) and tight junction (TJ) are seen at their luminal aspects (X 18000).

The Type II cells were rich in ribosomes, glycogen and inclusion bodies which were in different development stages. There were also a few mitochondria, well-developed Golgi complexes and granular endoplasmic reticulum in their cytoplasms (Figure 14). Some mature laminated inclusion bodies were found to be released from the apical surfaces of Type II cells (Figure 15). Surfactant attracted attention in the primitive alveolar spaces in the form of myelin figures (Figures 12, 13 and 15).

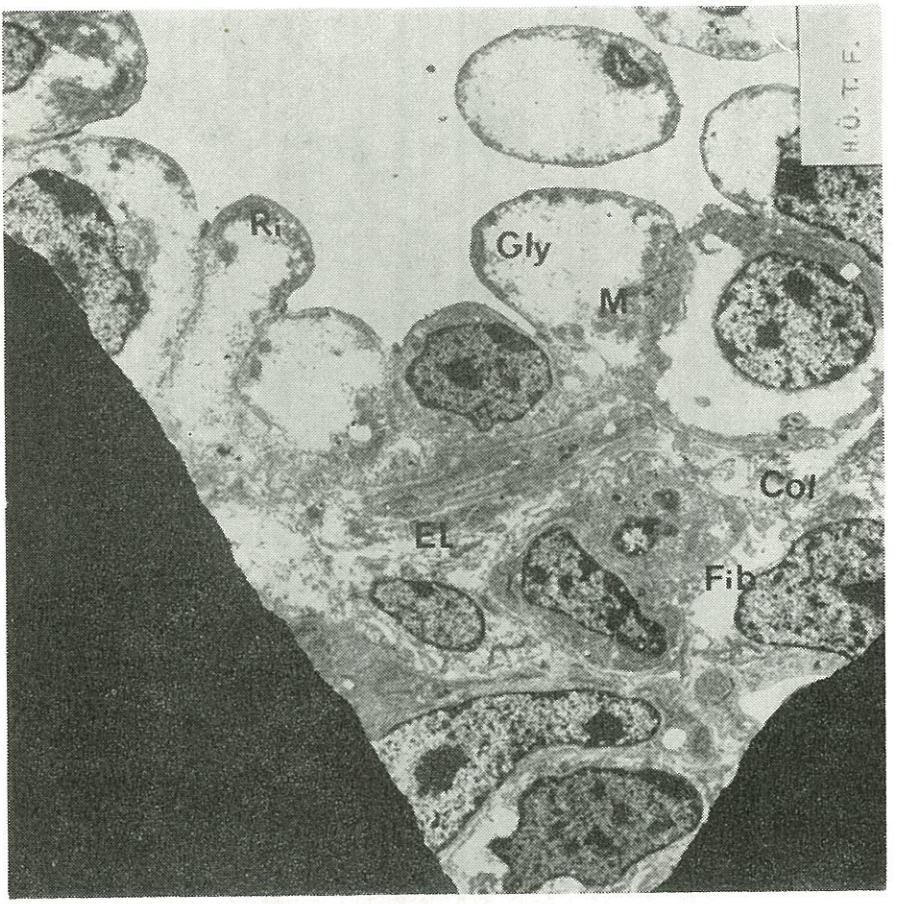

Figure 11

This is a primitive respiratory bronchiole in the 18-day-old mouse embryon. Cubic endodermal epithelial cells lined the primitive respiratory bronchiole contain prominent aggregates of glycogen (Gly), many ribosomes (Ri) and a few mitochondria (M). Intercellular fibrillar elements and fibroblasts (Fib) are seen around the primitive respiratory bronchiole (X 6600).

The Type I cells had large nuclei and a small amount of cytoplasms with well-defined cytoplasmic extensions. They contained scattered ribosom and glycogen, a few mitochondria and little endoplasmic reticulum. They played a big role in the formation of the blood-air barriers (Figure 16). There were also some cubic endodermal cells which were lack of inclusion bodies, poor of cytoplasmic organelles and rich in glycogen among the Type I and Type II alveolar epithelial cells (Figures 12 and 13).

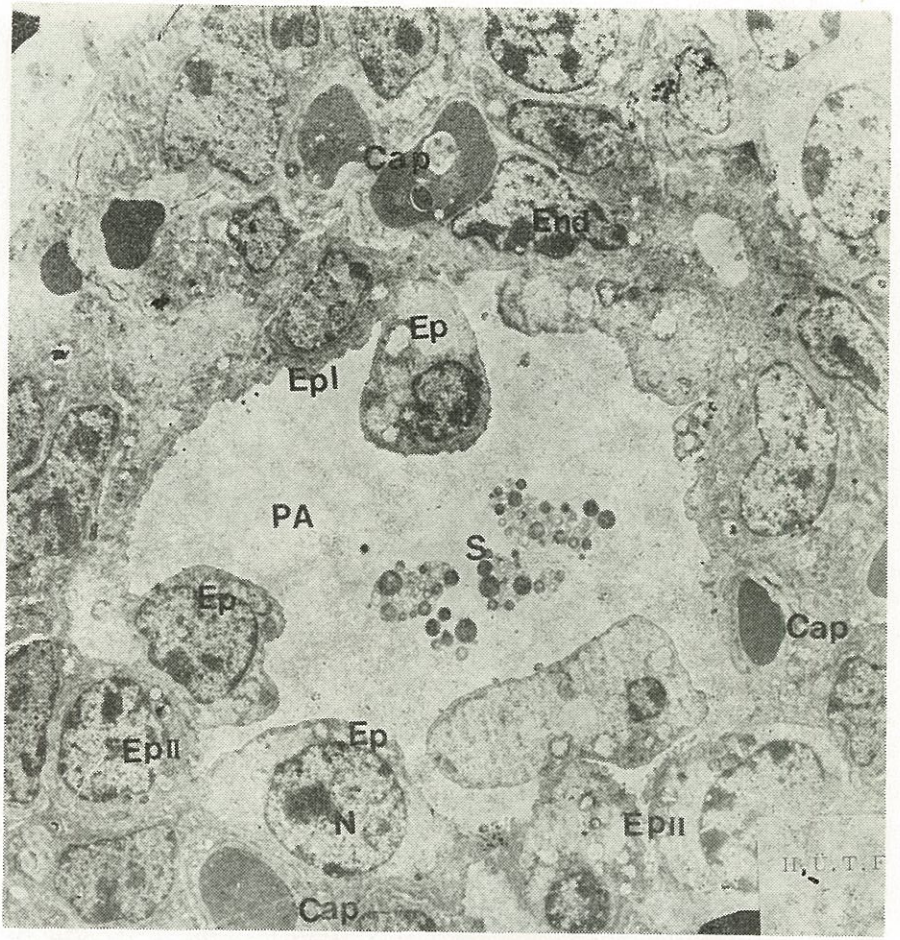

Figure 12

This is an electronmicrograph from the 19-day-old embryon. Primitive alveoli (PA), Type I (EpI) and Type II (EpII) endodermal epithelial cells, surfactant (S) and some undifferentiated endodermal epithelial cells (Ep) are observed (X 6600).

The mesenchymes, undergoing septation were composed of capillaries, undifferentiated mesenchymal cells and fibroblasts. Protofibrils, collagen fibrils and elastic fibers were secreted by fibroblasts into intercellular spaces (Figure 17).

20-21- day-old embryons: By the 20th and final day of gestation, the various types of cells lining conducting airways and alveoli could be readily distinguished (Figures 18 and 21). Bronchioles were covered by two major cell types; cilliated and non-cilliated cells (Figure 18).

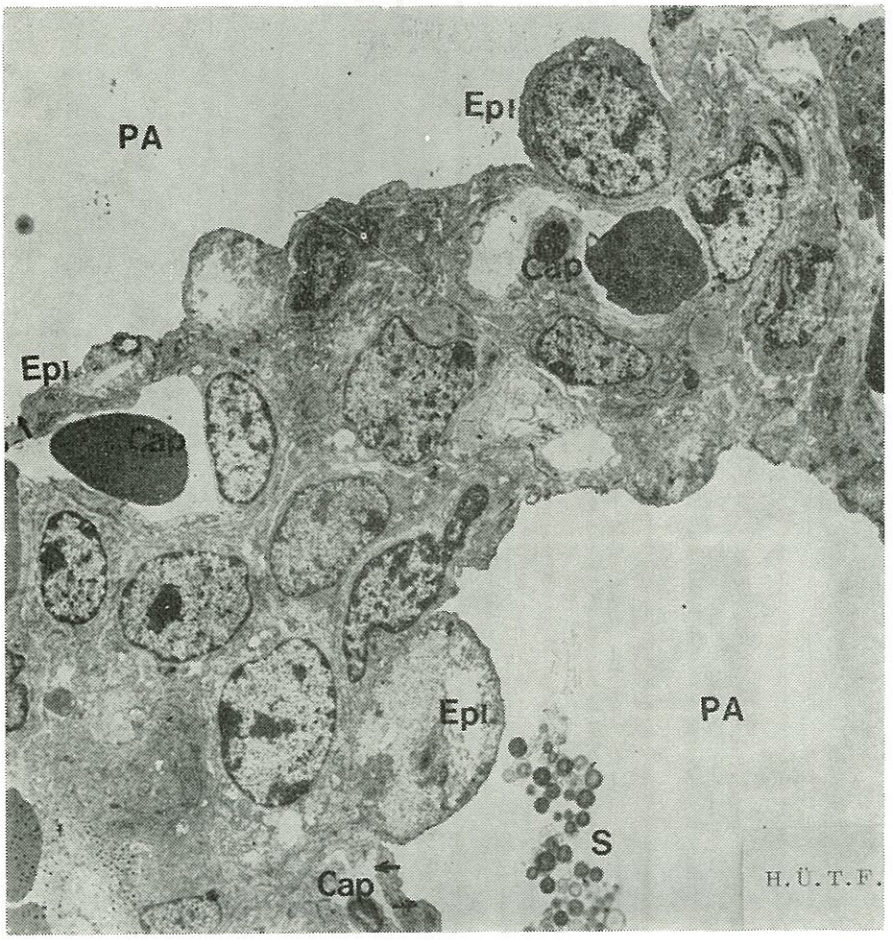

Figure 13

Wide alveolar septum, Type I endodermal epithelial cells (Epl), blood air barriers (arrows) and surfactant (S) in primitive alveolar space attract attention in the 19-day-old embryon (X 6600).

They were attached by junctinal complexes and had basal laminae (Figures 18 and 19).

The cilliated cells were columnar and few in number. They contained many ribosomes, mitochondria and variable amounts of granular endoplasmic reticulum with distended cisternae and prominent Golgi complexes (Figure 19).

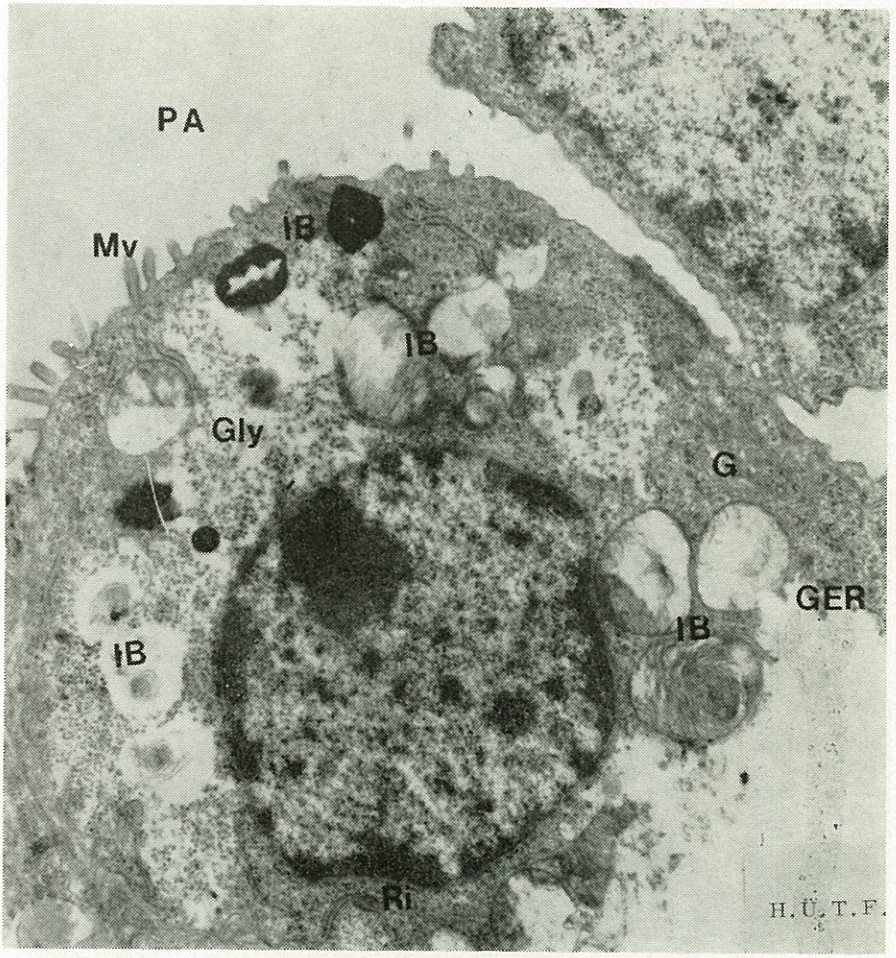

Figure 14

Type II endodermal epithelial cell (EpII) from the lung of the 19-day-old mouse embryon. It is rich in ribosomes (Ri), glycogen (Gly) and inclusion bodies (IB) which were in different development stages. A few mitochondria (M), well-developed Golgi complex (Go) and granular endoplasmic reticulum (GER) are seen in the cytoplasm of this cell. Its apical surface displays microvilli (Mv) (X 18000).

The non-cilliated cells were tall, dome shaped, protruding into bronchiolar lumina. They were full of glycogen granules but only thin cytoplasmic rims, containing cell organelles took place in the periphery of the cells (Figures 18 and 20).

Underneath the basal laminae of bronchiolar lining cells, there were loose and thin connective tissues consisting of undifferentiated cells, fibroblasts, secreting protofibrils, collagen fibrils and elastic fibers, and smooth muscle cells (Figure 18).

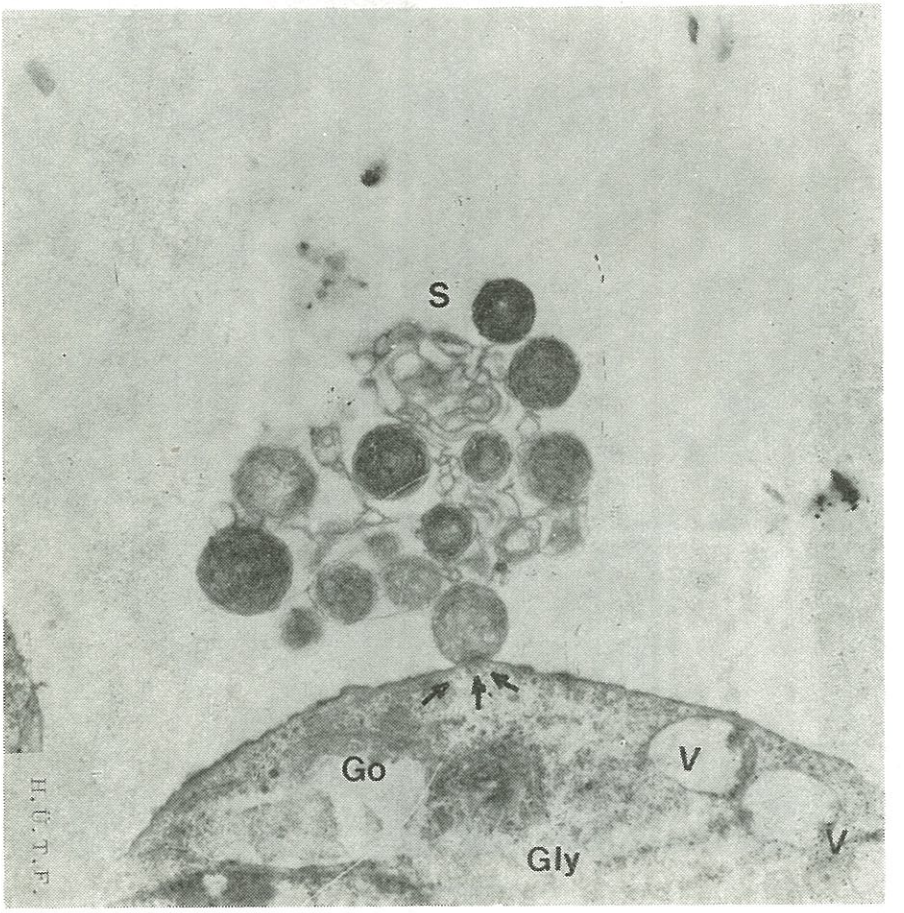

Figure 15

Surfactant (S) is released from Type II epithelial cell (EpII) in the 19-day-old mouse embryon. Glycogen (Gly), vacuoles (V) and Golgi complex (Go) are seen in the cytoplasm of this cell (X 18000).

The arterioles accompanying broncioles were seen. They were lined by low cuboidal epitheliums and surrounded by thin connective tissues, composed of fibroblasts, elastic fibers and collagen fibrils (Figure 18).

Alveoli attracted attention with their characteristic lining cells (Figure 21). The Type II cells had numerous laminated mature and immature inclusion bodies, ribosomes and glycogen. They also contained a few mitochondria, some granular endoplasmic reticulum and well-developed Golgi complexes (Figures 21 and 22).

The Type I cells, having attenuated cytoplasmas and flattened shapes, formed the blood-air barriers in association with capillaries. A few mitochondria, little endoplasmic reticulum, scattered ribosomes and glycogen were found in their cytoplasms (Figures 21 and 22).

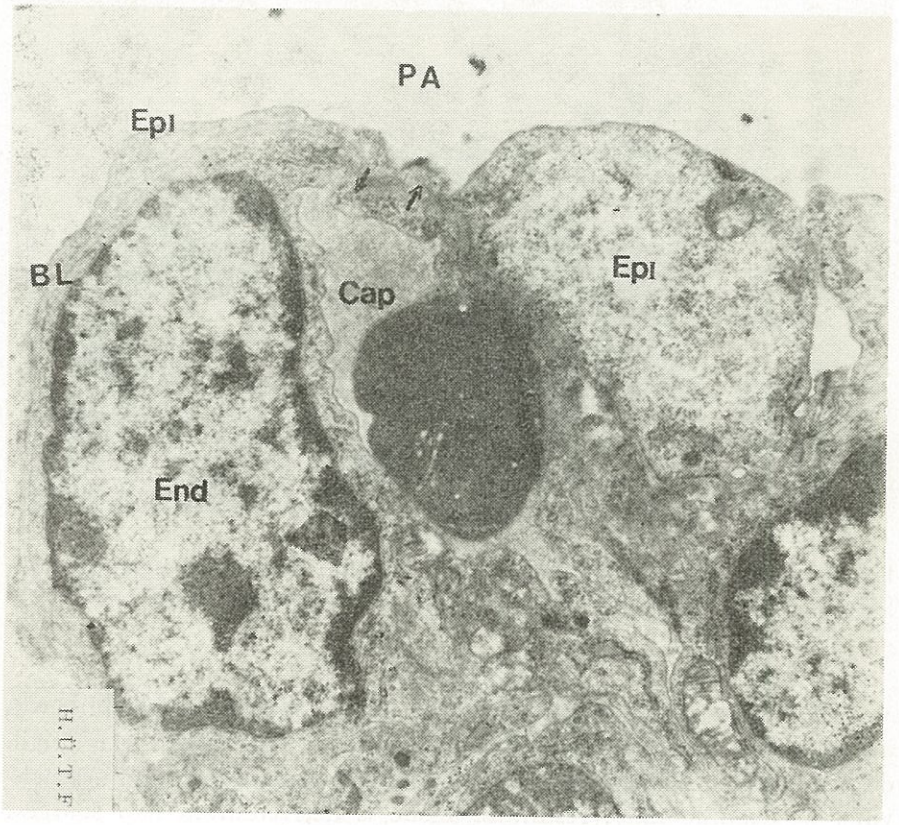

Figure 16

The cytpolasmic extension of Type I endodermal epithelial cell (EpI), its basal lamina (BL) and the cytoplasmic extension of endothelium (End) of capillary are observed to form the blood-air barrier (arrows) (X 18000).

Protofibrils, collagen fibrils, elastic fibers, fibroblasts, and undifferentiated mesenchymal cells were noticed in the alveolar septums. The thickness of these septums varied from place to place (Figure 22).

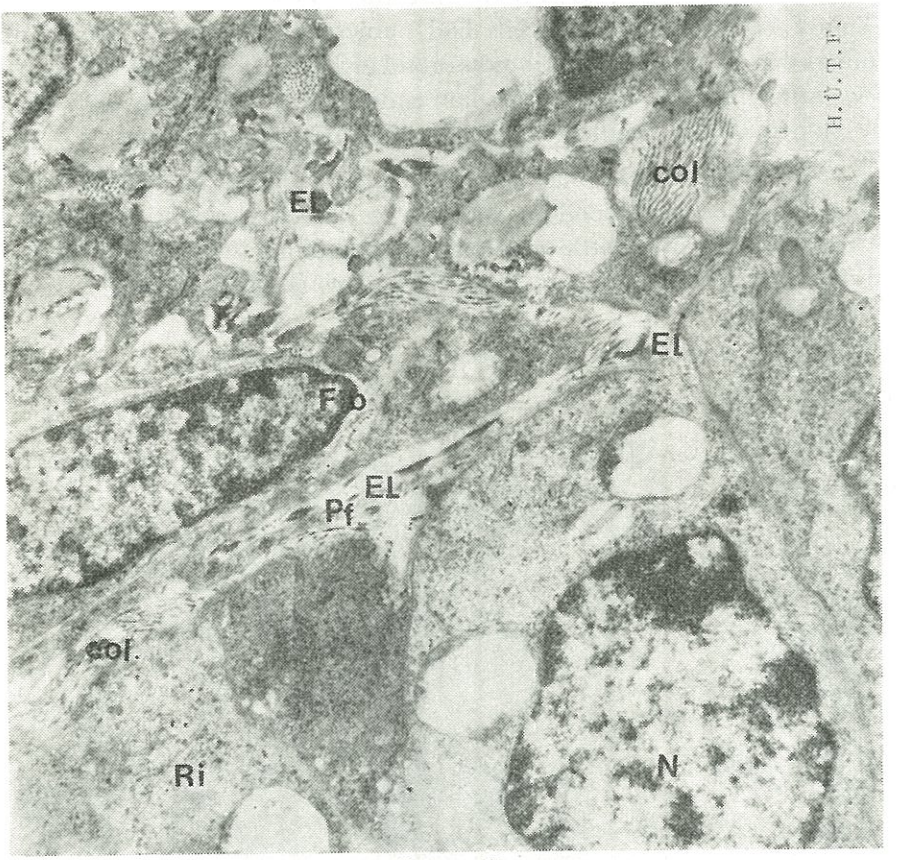

Figure 17

Protofibril (Pf), collagen fibrils (Co) and elastic fiber (El) released from fibroblast (Fib) attract attention in the alveolar septum of the 19-day-old embryon (X 18000).

Discussion

One of the early investigation upon the development of the lung was an extensive one by Flint,[10] who described in some detail the origin of the endodermal buds and their branching in association with mesenchyme. Developmentally, respirator tissue is derived from two sources: endoderm and mesenchyme. Cooper,[11] reported three generations of branches of the endodermal lung bud. The first gives rise to non-respiratory bronchi, bronchioles and terminal bronchioles, the second to alveolar ducts and the third to alveolar sacs and alveoli.

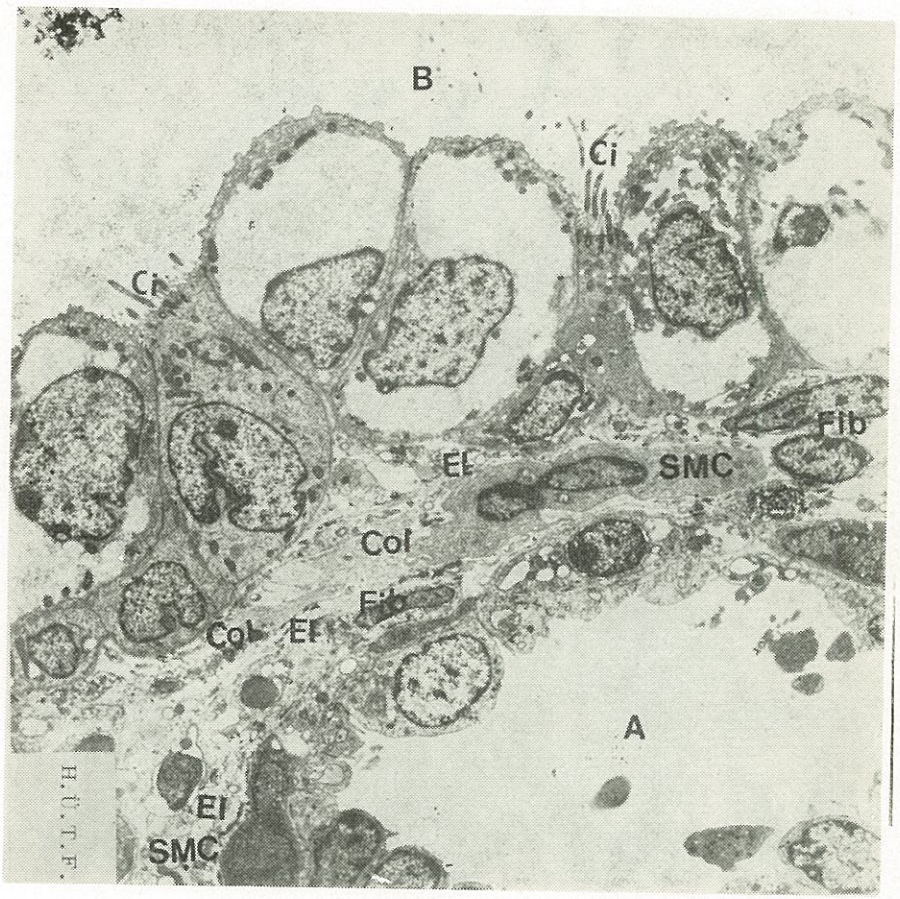

Figure 18

Cilliated (Ci) and non-cilliated cells lining the bronchiole (B), and an arteriole (A) are seen in the 20-21 day-old embryon. Loose connective tissue composed of smooth muscle cells (SMC), fibroblasts (Fib), collagen fibrils (Col) and elastic fibrils (El) are observed underneath the basal laminae of the bronchiole and the arteriole (X 6600).

Connen and Balis[5] investigated branching of endodermal bronchial tree into three periods of developing lung. In glandular period, by dichotomous branching, the primitive bronchi generate other primitive bronchi and bronchioles. In canalicular period, all generations of primitive bronchi develop and by dichotomous branching, primitive bronchi generate primitive respiratory bronchioles. In alveolar period, primitive respiratory bronchioles generate other respiratory bronchioles as well as alveoli.

According to Robert Rugh[12] observations, a pair of lung buds develop in the 10-day-old mouse embryons. The right lung bud is slightly

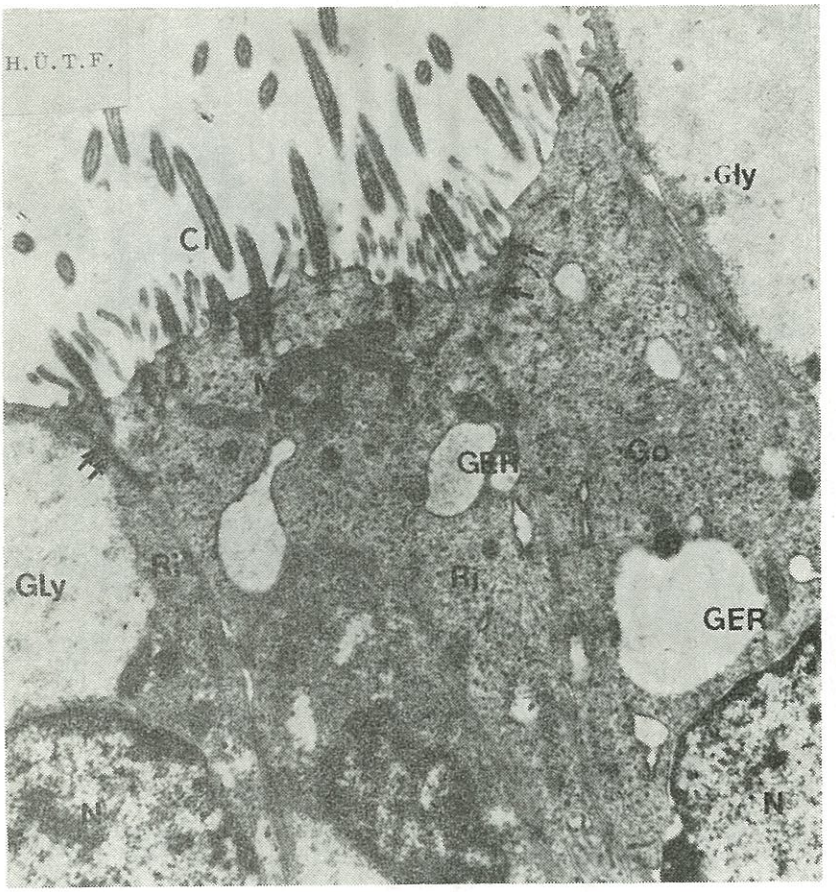

Figure 19

Cilliated cells (Ci) contain many ribosomes (Ri) and mitochondria (M) and variable amounts of granular endoplasmic reticulum (GER) with distended cisternae and prominent Golgi complex (Go). They are attached to each other and to non-cilliated cells by junctional complexes (arrows) (X 18000).

in advance of the left on day $10^1/_2$. Bronchi are identified by day 11. Bronchi have 2 or 3 buds in the $11^1/_2$-day-old embryons. On day $12^1/_2$, the right bronchus has both secondary and tertiary buds (with fewer in the left) In 12-day-old embryons, bronchial tree has many branches and buds. By $14^1/_2$ day, the bronchial tree branches further and lung is lobed and vascularized.

In the present study, the branching of the bronchial tree was observed according to glandular, canalicular and alveolar periods in the lungs of developing mouse embryons. The lungs of the 12, 16 and 17-day old embryons had glandular periods. The lungs of the 12-day-old

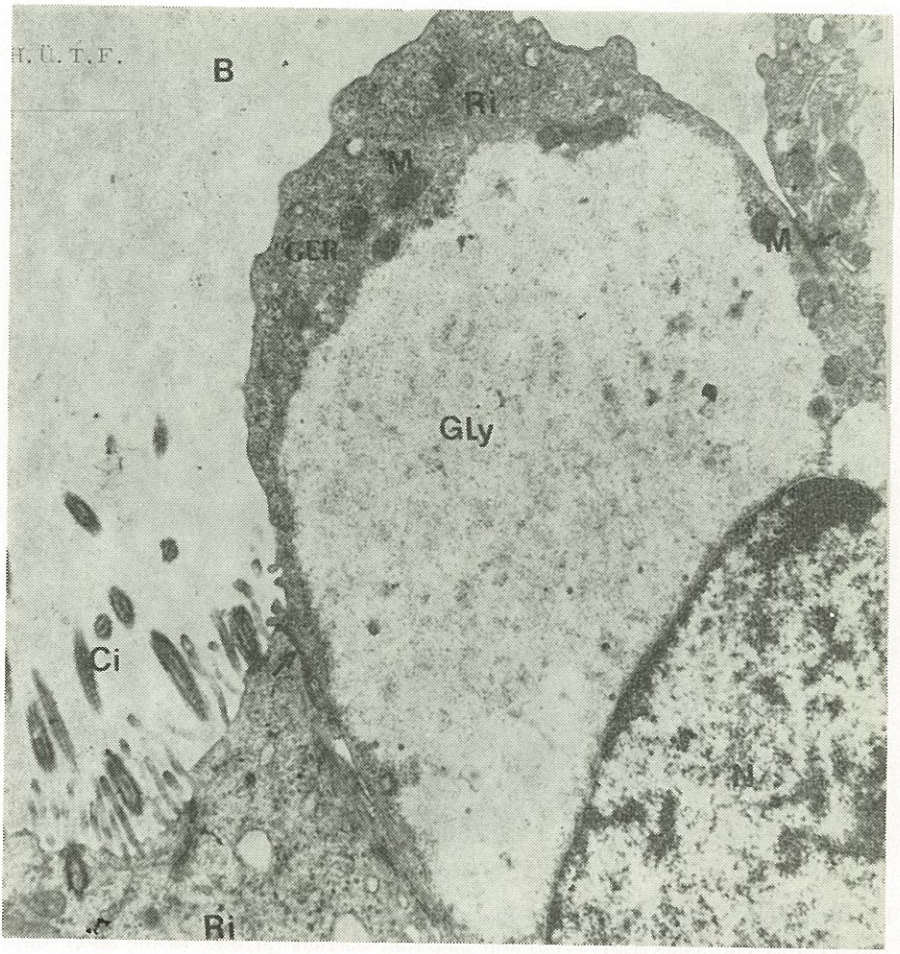

Figure 20

Non-cilliated cells are full of glycogen (Gly) granules, but only thin cytoplasmic rims containing a few mitochondria (M), small amount of granular endoplasmic reticulum (GER) and many ribosomes (Ri) take place in the periphery of the cells. They have well-developed nuclei (N) and are attached to each other and to cilliated cells by junctinal complexes (arrows) (X 18000).

embryons contained primitive bronchi which were lined by stratified columnar epitheliums. Bronchioles which were branches of the primitive bronchi and covered by prismatic epitheliums, attracted attention in the lungs of the 16, 17, 18, 19 and 20-21-day-old embryons.

Canalicular period were seen in the lungs of the 18 and 19-day-old mouse embryons. In this period, the primitive respiratory bronchioles were lined by cubic epitheliums.

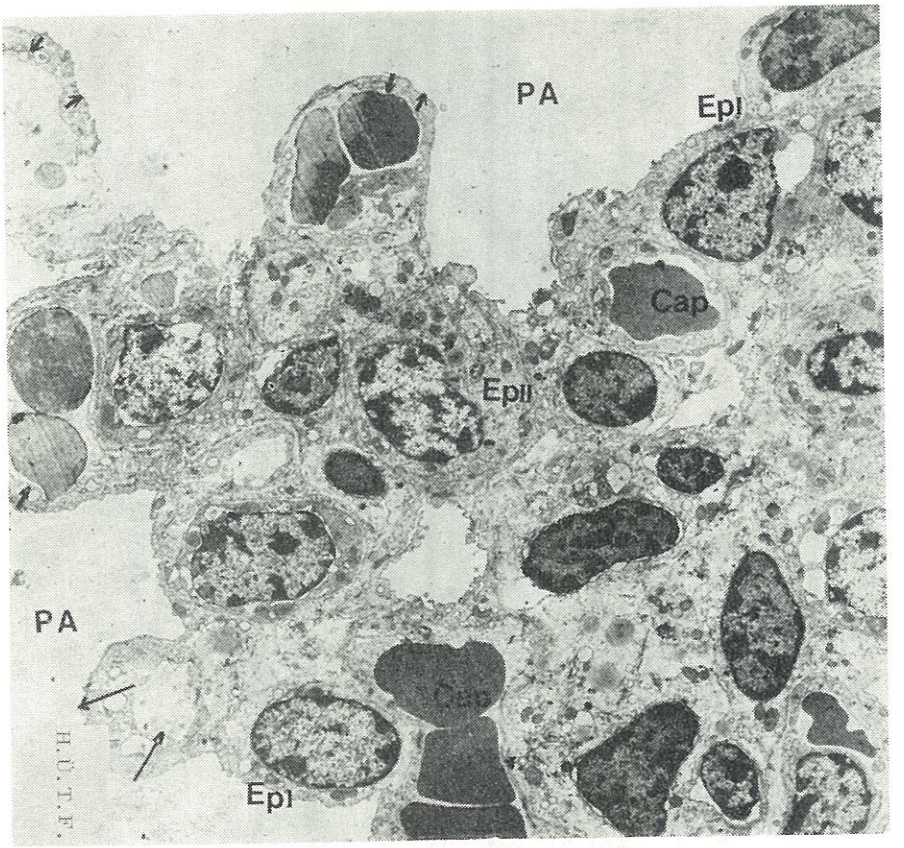

Figure 21

Primitive alveoli (PA) attract attention with their characteristic lining Type I (EpI) and Type II epithelial (EpII) cells and blood air barriers (arrows) in the 20-21 day-embryon (X 6600).

The lungs obtained from 19-day-old up to full term showed alveolar period. This period were characterized by the alveoli which were final branches of bronchial tree and encircled by flat and cubic endodermal epitheliums.

Woodside and Dalton,[13] Buchingam and Avery,[14] and Schneeberger[15] in mice; Leeson and Leeson[6]; Buchingam[16] et al; Balis and Connen[17]; Kikkawa et al[18] and Noak[8] in rabbits; Banks[19] in hamster; Kikkawa[20] in Lambs; and Petrik[7] in chicken investigated general ultrastructural features of developing lungs. They did not direct their studies toward the ultrastructural differentiation of the endodermal cells in the conducting air ways during the lung development.

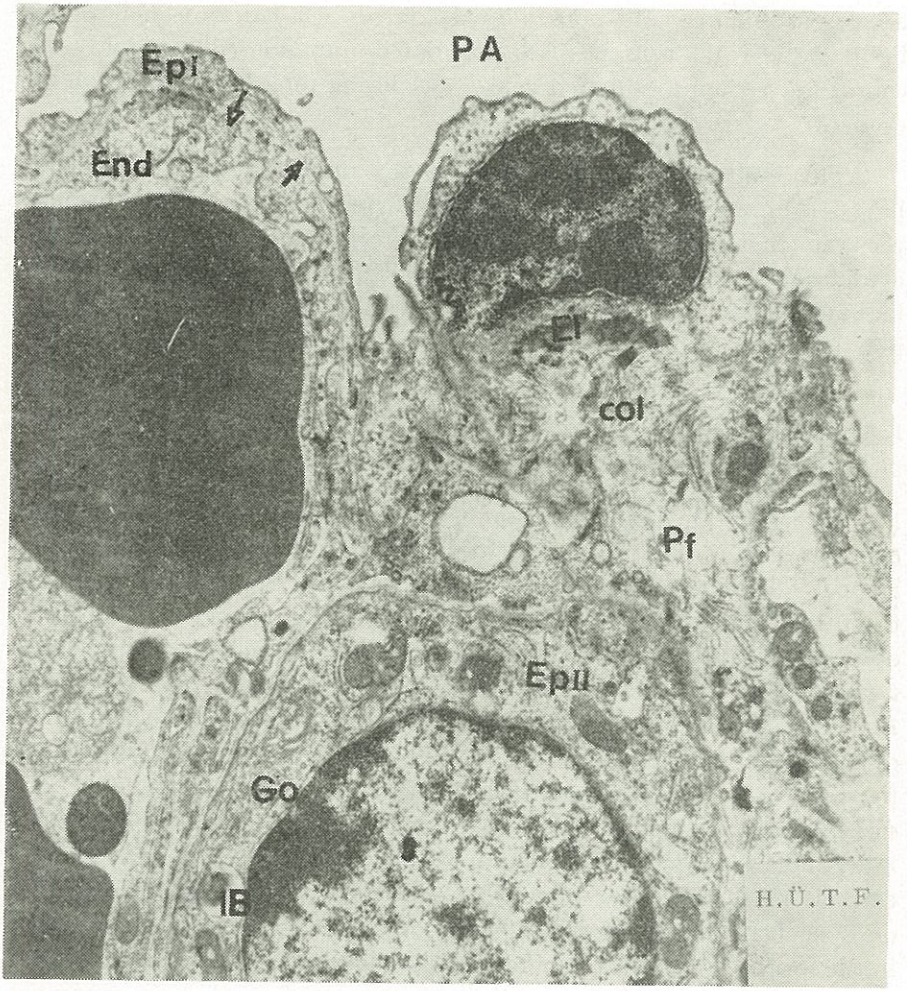

Figure 22

Protofibrils (Pf), collagen fibrils (Col) and elastic fibres (El) spread throughout the primitive alveolar zone in the 20-21 day-old mouse embryons. The extension of Type I (EpI) epithelial cell, blood-air barrier (arrows) and Type II epithelial cells (EpII) are observed. (X 18000).

In this study, the primitive bronchi lined by stratified columnar endodermal epitheliums, displayed dark and light cells according to their organelles in the lungs of the 12-day old mouse embryons. As development proceeds and the bronchial tree ramifies, conducting air ways were covered by prismatic epitheliums. Light and dark endodermal cells were identified very easily from the beginning of the 17-day up to the 19-day old mouse embryons. In the 19-day-old embryons, dark and light cells started to

appear as cilliated and non-cilliated. These facts led us to believe that light endodermal cells were differentiated into non-cilliated or Clara cells, dark cells into cilliated cells, considering their morphologic development.

The epitheliums which lines the alveoli in mature mammalian lung is composed of two types of cells. Type I cells,[21] or membranous pneumonocytes[22] have a small, squamous cell body with an attenuated cytoplasm covering most of the alveolar surface. Type II cells,[21] the granular pneumonocytes[22] are cuboidal and contain characteristic cytoplasmic inclusion bodies.

The endodermal origin of the alveolar epitheliums has been established by many workers studying on lung development in vitro,[23, 24, 25, 26, 27] and at ultrastructural level.[7, 17, 18, 28, 29, 30]

For years the differentiation of the Type I endodermal epithelial cells has been a matter of controversy. Balis and Conen,[17] suggested that Type I cells derive from Type II cells. Petrik[7] who studied the ultrastructure of the chicken lung in the final stages of embryonic development thought that Type I cells arise secondarily from granular pneumonocytes by losing characteristic osmiophilic inclusions and organoids. According to Kikkawa et al[18] findings, both types of cells start to be differentiated from endodermal epithelial cells as two types of cells when the volume of the lung's potential air space rapidly increases. Once the differentiation is made they do not transform into one another. Several independent observations related to the repair of damaged alveolar epithelium showed that in oxygen poisoning the squamous processes of Type I alveolar epithelial cells become damaged and a repair ensus in the form of a lining of cuboidal cells which have all the characteristics of Type II cells; these are subsequently transformed into squamous Type I cells.[31, 32, 33]

Evans et al[34] have demonstrated in an autoradiographic study on tissue repair after NO_2-damage, that only Type II cells entered the division cycle by synthesizing DNA, whereas Type I cells did not; they also showed that labeled Type I cells could be found within 48 hours presumably arising from labeled Type II cells.

Recently, Kayfman et al,[35] have claimed that Type II cells might represent the stem cell of the Type I epithelial cell, because Type I cells increased in number without occurrence of any 3 H-thymidine labeling (DNA Synthesis) in the nuclei of these cells. It has been thought that the reason for the inability of Type I cells to divide probably lay

in their unusually high level of topological differentiation in that each cell formed multiple apical cytoplasmic plates, a configuration which make cytoplasmic division difficult.[36, 37]

In this study, the lungs of the 19 and 20-21 day old mouse embryons displayed alveolar period. The walls of the primitive alveoli in this period were lined by two types of cells. The cells which played a big role in the formation of blood-air barriers, had large nuclei, a small amount of cytoplasms with well-defined cytoplasmic extentions and were poor of cytoplasmic organelles, were Type I cells or membranous pneumonocytes. The cells which were cubic in shape, rich in characteristic inclusion bodies and glycogen, were Type II or granular pneumoncytes. The cubic endodermal cells which were observed in the canalicular period of the lungs of the 18 day-old mouse embryons started to be differentiated in the alveolar period. Some of these cubic cells were probably differentiated into Type I cells when the capillaries established direct contact with them or, in another word, when the endothelial cells of capillaries affected on them inductively. Some of them were differentiated into Type II cells containing characteristic inclusion bodies. Some cubic endodermal epithelial cells remained undifferentiated form among the Type I, and Type II cells. These fact led us to consider that the cubic endodermal cells which lack of inclusion bodies, rich in glycogen, poor of cytoplasmic organelles, were probably stem cells of the Type I and II cells in the immature or developing mouse lung.

Inclusion bodies were first described by Sjostrand and Sjostrand[38] and demonstrated electron microscopically by Sclipköter,[39] and Low.[40] Karrer[21] found the inclusions to be present in the Type II alveolar cells. Mature inclusion bodies have generally been identified as concentrically laminated figures in the Type II cells of various mammalian lungs. Before maturation, they displayed many transitional forms in the developing process.[16, 17, 18, 20, 41-45]

The inclusion bodies in Type II cells are thought to be related to pulmonary surfactant (surface active-antiatelectatic material), because surfactant can first be detected in fetal lungs when there are significant number of Type II cells with inclusion bodies. Inclusion bodies and surfactant both appeared in fetuses about the 18th day of gestation in mice, term 21 days;[14] 27th day in rabbit, term 30-31 days;[18] and 121 to 126 days in lambs, term 147 to 150 days.[20]

With routine electron microscopic techniques material related to surfactant is seen in alveolar spaces in the form of myelin figures which have the structural features of phospholipids.[18, 20, 42-57]

Maclin[22] suggested that Type II alveolar epithelial cells secrets granular substances into alveoli. Bencsh[58] reported that the lamellar bodies of granular pneumocytes are released into the alveolar lumen. But, he did not describe the mode of secretion. Balis and Connen[17] found that the inclusion bodies are extruded by evagination of their membrane from the cytoplasm of Type II cells into alveolus. This findings have been confirmed by others.[18, 20, 59-60] Sun[42] observed that the osmiophilic inclusions may extrude through a channel formed within the cell or by rupturing the cell membrane. Hatasa and Nakamura[61] suggested that lamellar bodies are released into alveolar space by merocrine process. He observed that the limiting membrane of the inclusion bodies come into contact with the apical plasma membrane of the Type II cells; disappears at the place of contact and the contents of the inclusion bodies become continuous with alveolar space.

In the present study, the inclusion bodies in Type II cells and surfactant in the form of myelin figures in the alveolar spaces first started to appear in the lungs of the 19-day-old mouse embryons, term 21 days. Type II cells contained both mature, concentrically laminated inclusion bodies and immature, transitional forms at various developing stages. The laminated mature inclusion bodies which were being extruded from Type II alveolar cells, were not encountered. Free myelin figures and the myelin figure which was still contact with the apical membrane of Type II cells, were observed in alveolar spaces of the lungs of the 19, and 20-21-day-old developing mouse embryons.

Elastogenesis in the developing normal lung has been extensively studied under the light microscope.[62, 63] Jones and Barson[64] investigated the process of elastogenesis in the chick lung at the electron microscopical level. Recently, Collet and Desbiens,[65] studied on the fine structure of myogenesis and elastogenesis in the developing rat lungs throughout the 15th to the 21st days of the gestation period. They observed that myogenesis follows bronchial development and stops at the beginning of the alveolar zone, at the primitive respiratory bronchial level. Primitive respiratory bronchioles are visible on the 19th and are characterized by an early elastogenesis carried out by fibroblast. At this stage there are no elastic fibers around the alveolar tubulus. Then (20th and 21st days) elastogenesis spreads throughout the alveolar zone, accompanying the alveolization process. Myoblast and fibroblast originate from the same primitive mesenchymal cells.

In this study, in granular period, some undifferentiated mesenchymal cells accumulating around the bronchioles in the 16 day-old embryons,

were differentiated into smooth muscle cells to form future peribronchial muscular coats. In canalicular period, vascular mesenchymes displayed wide septation because of the primitive respiratory bronchioles. In these septums, some undifferentiated mesenchymal cells were differentiated into fibroblasts which started to release the protofibrils, collagen fibrils and elastic fibres. The elastogenesis and collagenogenesis were carried out by fibroblast and took place at the same time.[66] In 19 and 20-21 day old embryons (in alveolar period) elastogenesis and collagenogenesis spread throughout the alveolar zone, accompanying the alveolization process. Around the conducting air ways, loose and thin connective tissues consisting of undifferentiated cells, fibroblasts secreting protofibrils, collagen fibrils and elastic fibres were also observed.

Summary

The structural differentiation in the lungs of the 12, 16, 17, 18, 19 and 20-21-day-old mouse embryons were investigated under the electron microscope. Three periods were established during the genesis of mouse lung. They were glandular, canalicular and alveolar periods. Light and dark prismatic endodermal epithelial cells lining the glandular tubes of lungs in glandular period were differentiated into non-cilliated and cilliated cells covering conducting air ways in canalicular and alveolar periods. Primitive alveoli were lined by Type I or membranous pneumonocytes and Type II or granular pneumonocytes. They originated from cuboidal endodermal epithelial cells observed in canalicular and alveolar periods. Inclusion bodies in Type II cells and surfactant in the form of myelin figures in the alveolar spaces first attracted attention in the lungs of the 19-day-old mouse embryons. Myogenesis was first bserved in glandular period (16 day old embryons). Elastogenesis and collagenogenesis, carried out by fibroblasts and taking place at the same time, first started to appear in the 18-day-old embryons.

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Biomechanics of Total Knee Replacement

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The knee joint probably plays the most important role in human locomotion. Its construction and performance during various types of lower limb activity such as walking, running, climbing, or kicking demonstrate one of the marvelous achievements of nature. To understand fully everything associated with the knee joint is certainly not an easy task. It requires the combined efforts of those who are trained in the disciplines of anatomy, biochemistry, kinesiology, mechanics (including materials, dynamics, and fluid mechanics), medicine, pathology, physiology, and surgery with special emphasis on orthopaedic surgery.

In a recent paper this author[1] discussed in some detail osteoarthritis and biomechanics of tibial osteotomy. This paper is intended to be a follow-up to that paper and serve two additional purposes, namely, to present a critical review of the knee arthroplasty devices and the biomechanics aspects of the design of a total knee prosthesis. The paper will be concluded with a report on the geometric knee prosthesis.

CRITIQUE OF WIDELY USED KNEE ARTHROPLASTY DEVICES

There are various knee arthroplasty devices that are available for the surgical treatment of advanced stages of rheumatoid arthritis or osteoarthritis involving both compartments of the knee joint. The classification of these devices can be made on the basis of whether they are hemi-arthroplasty or total arthroplasty devices; whether they are hinged or non-hinged; whether they are duo-compartmental or uni-compartmental devices. Following is a brief decscription of these devices and their major advantages, as well as disadvantages.

Hemi-arthroplasty or Interpositional Devices: These devices are intended to replace the femoral or tibial articulating surfaces by

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either polymers or metals. For example, the MGH (Massachusetts General Hospital) knee is a metallic femoral condyle replacement device with an intramedullary stem for fixation. It is simply a metallic replica of the distal femur. In the MacIntosh prosthesis either or both of the tibial plateaus is relined by a slightly concave stainless steel disc which is in contact with the femur (Figure 1). The MacIntosh prosthesis is designed to be anchored in a shallow depression cut in the tibial plateau and its motion is prevented by a rim of bone surrounding the prosthesis. The McKeever and Sbarbaro prostheses are very similar to MacIntosh except their bottom surfaces are modified for better fixation. The shortcomings of hemi-arthroplasty are: a) wear of the prosthesis if polymers are used or wear and degradation of the mating bone surfaces if metals

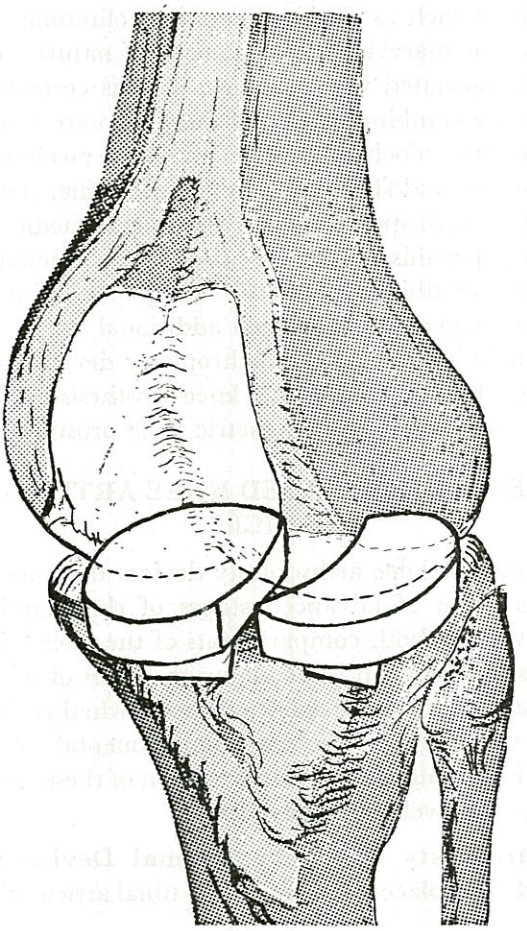

Figure 1
Schematic drawing of a hemi-arthroplasty device (McKeever tibial plateaus)

are used; b) the surgical difficulties of anchoring the prosthetic components (MacIntosh); c) possibility of persistence or reappearance of disease in the joint; and d) "right" and "left" versions are required (MGH, McKeever).

Hinged Prosthetic Devices: These prostheses are total knee replacement devices which flex about a single axis and are anchored in the bone cavities by intramedullary stems either with or without acrylic cement. Walldius, Shiers, Herbert, Young, LaGrange-LeTournel, Stabilo-Condylar, Stanmore, Sheffield, Gebhardt, and Guepar prostheses are examples of hinged prostheses (Figure 2). The major disadvantages of hinged prostheses are: a) the normal kinematics of the knee joint cannot

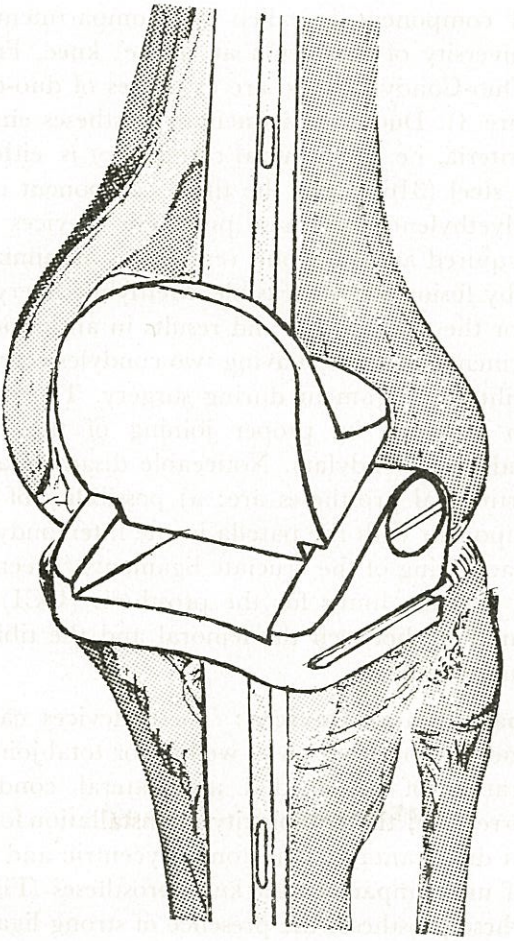

Figure 2
Schematic drawing of a hinged prosthesis (Walldius knee)

be reproduced due to a fixed axis of flexion; b) it requires a large amount of bone removal, thus, making the salvage operation very difficult if not impossible (Walldius, Shiers); c) due to the constant sliding nature of the hinge, the joint may be subject to excessive wear and fretting corrosion; d) some designs indicate a partial or complete patellectomy (Shiers); and e) both "right" and "left" versions must be supplied for the ones with long stems. The primary advantage of a hinged prosthesis is its potential to restore function to severely degradated knees without the presence of the ligaments. In addition, for some designs the cement requirement is also removed (Walldius).

Duo-Compartmental Devices: A prosthetic device which has both the medial and the lateral condylar surfaces united in a single femoral or tibial component is called duo-compartmental. Geometric knee, UCI (University of California at Irvine) knee, Freeman- Swanson knee, and Duo-Condylar knee are examples of duo-compartmental prostheses (Figure 3). Duo-compartmental prostheses employ metal on plastic design criteria, i.e. the femoral component is either chrome-cobalt or stainless steel (316L) and the tibial component is UHMWP or high-density polyethylene. For these prosthetic devices no medullary penetration is required and the bone removal is minimized; thus, salvage operation by fusion is quite feasible. Methylmethacrylate is used as a binding agent for the components and results in an immediate fixation. In duo-compartmental designs, having two condyles connected together on one part facilitates alignment during surgery. The retention of the cruciates is also achieved by proper joining of the condyles (Geometric, UCI and Duo-Condylar). Noticeable disadvantages of some of the duo-compartmental prostheses are: a) possibility of interference of the femoral component with the patella in the intercondylar area (Duo-Condylar); b) sacrificing of the cruciate ligaments (Freeman-Swanson); c) lack of axial rotation limits for the prosthesis (UCI); and d) wear debris can accumulate between the femoral and the tibial components (Geometric, Duo-Condylar).

Uni-Compartmental Devices: These devices can be used for single compartment reconstruction as well as for total joint replacement. Although separation of the medial and lateral condyles facilitates varus/valgus correction, the complexity of installation for the total joint replacement is a disadvantage. Gunston Polycentric and Marmor knees are examples of uni-compartmental knee prostheses (Figure 4). Needless to say, for these prostheses the presence of strong ligaments is essential. Retention of the cruciate ligaments and minimum amount of bone removal are some additional advantages of the uni-compartmental de-

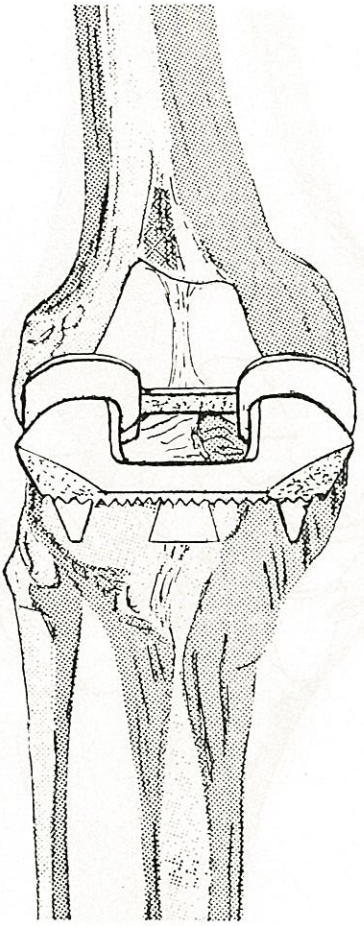

Figure 3

Schematic drawing of a duo-compartmental device (Geometric knee prosthesis)

vices. Some individual disadvantages of these devices are: a) very limited bearing area is provided (Polycentric); b) installation is complex (Polycentric); c) wear debris can accumulate on the tibial component (Polycentric); and d) positioning instrumentation is lacking (Marmor).

DESIGN CRITERIA OF A TOTAL KNEE PROSTHESIS

At this time, it seems important to take a fresh look at knee arthroplasty and provide the design criteria for a total knee prosthesis. The principal goals of any knee arthroplasty procedure are the relief of pain, improvement or restoration of function and stability. To achieve these goals a preliminary set of design criteria is presented below.

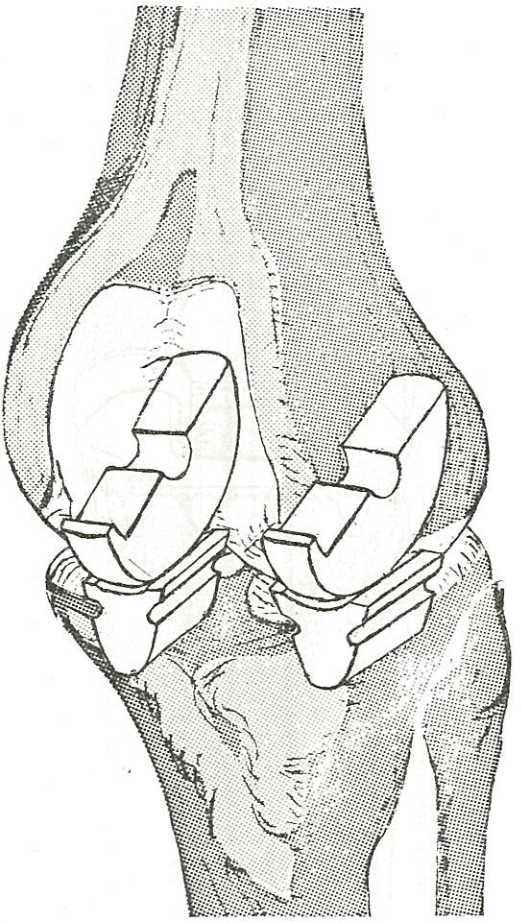

Figure 4
Schematic drawing of a uni-compartmental device (Gunston polycentric knee prosthesis)

1. Functional criteria for joint restoration should include:
   a. greater than 90° flexion
   b. complete extension with a locking-action
   c. axial rotation of the tibia relative to the femur about 9 degrees.
   d. stability under realistic biodynamic loads
   e. the repair potential of varus and valgus deformity.
2. The design should allow the retention of the cruciate and collateral ligaments. Furthermore, it should provide a smooth articulation for the patella and a patellar prosthesis for degenerated patellar cartilage.

3. The design should avoid extensive intra or extra medullary penetration and sharp edges close to the skin surface.
4. The design must include all procedures and instruments required for quick and accurate installation. This feature will reduce time under anesthesia and guarantee optimal placement.
5. Less than 2.5 cm bone should be removed to allow salvage operation through arthrodesis.
6. Useful life of prosthesis should exceed the patient's life. This may be achieved through good fixation, low wear, low friction, and biocompatibility.
7. A user's manual for surgeons and patients should include:
   a. indications and contraindications
   b. instructions for surgeons, e.g., how to find optimal placement, how to implant at optimal placement, mitigating circumstances, assumptions
   c. educational materials for the patient which include anatomical diagrams of appropriate areas to explain the procedure and a discussion of pros and cons leading to reasonable expectations and limitations on activities.
8. Manufacturability is an imperative. Any successful design must include the ability to manufacture the device consistently and profitably with present day technology.

COMPARISON OF GEOMETRIC KNEE PROSTHESIS WITH THE NATURAL KNEE

This paper will be concluded with a report of a recent research of this author on the biomechanical comparison of a geometric knee prosthesis with the natural knee. The contraindications for tibial osteotomy are usually reasonable indications for geometric total knee replacement arthroplasty. Very advanced stages of rheumatoid arthritis or osteoarthritis involving both compartments of the knee joint, tibial plateau, or femoral mold arthroplasties that have failed to provide stability, mobility, and pain relief are indications for the geometric total knee replacement.

The investigation conducted on the geometric knee is a natural extension of our previous work[2, 3, 4] in understanding the biomechanics of the human knee joint. To study and compare the geometric knee prosthesis with the natural knee, a loading apparatus which allows the testing of the knee joint in various flexed and loaded positions of the

knee was designed and constructed. The foot of the specimen is attached to a force and moment measuring device (FMMD) located at one end of the apparatus and the pelvis of the specimen is attached to a loading screw located at the opposite end of the apparatus. The motion of the knee is constrained by two force transducers along anteroposterior and mediolateral directions during the flexed positions of the knee. These force transducers are used on the patella and either on the medial or lateral side of the femoral condyle depending upon the test specimen and the magnitude of the flexion angle. For the flexed and the loaded configuration of the knee, the constraining patella transducer serves in an equivalent manner as if the quadriceps femoris were present to maintain the equilibrium. The patella transducer provides the equivalent patellofemoral joint force which arises from the combined effects of forces acting on the patella ligament and quadriceps tendon.

Three cadaver legs were prepared by casting the ilium in between two aluminum channels approximately 12 cm apart for the purpose of attaching the pelvis of the specimen to the loading screw. All three test specimens were tested for the locked position as well as 25°, 40°, 70° and 100° flexed positions of the knee. One of the specimens was later im-

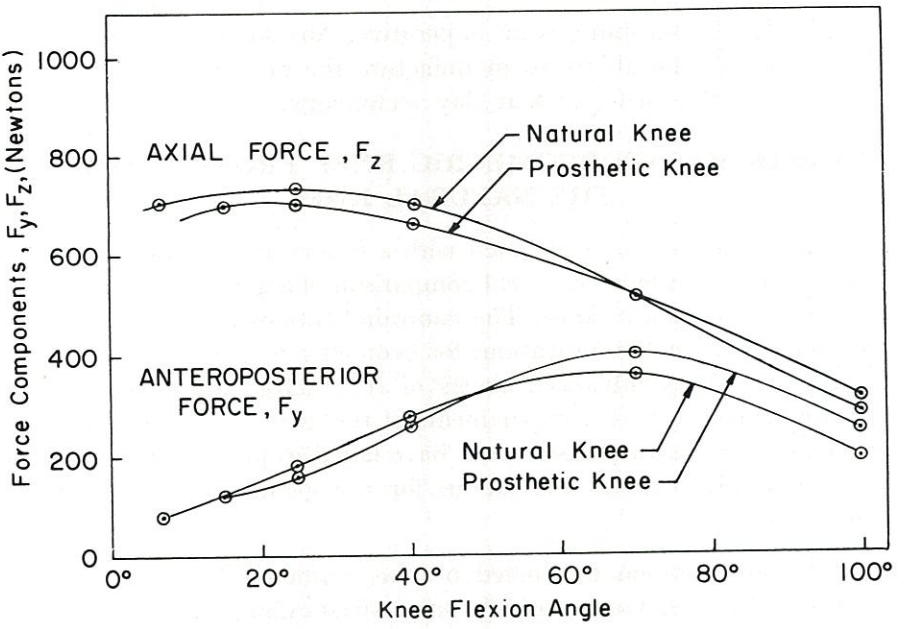

Figure 5

Comparison of force components at the knee center between the natural and prosthetic knee for various flexion angles with 800 Newtons (81.6 kg) of applied pelvic load.

planted with a geometric knee prosthesis and the same tests were repeated. A typical test consisted of applying up to 1000 Newtons (1 N. = 0.102 kg) of force, in eight or nine not necessarily equal steps, via the loading screw attached to the hemi-pelvic cast. The outputs of the six force transducers of FMMD and the outputs of two knee constraint transducers along with the coordinates of a reference point, designated by a nail head situated on the anterior poximal site of the tibia, were recorded for each pelvic load. All the experimental data were tabulated in 26 tables, each containing approximately 150 data points.

The theoretical analysis of the experimental data yielded results which are in the form of three-dimensional force and moment vectors at the knee center for various flexed positions of the knee and axial pel-

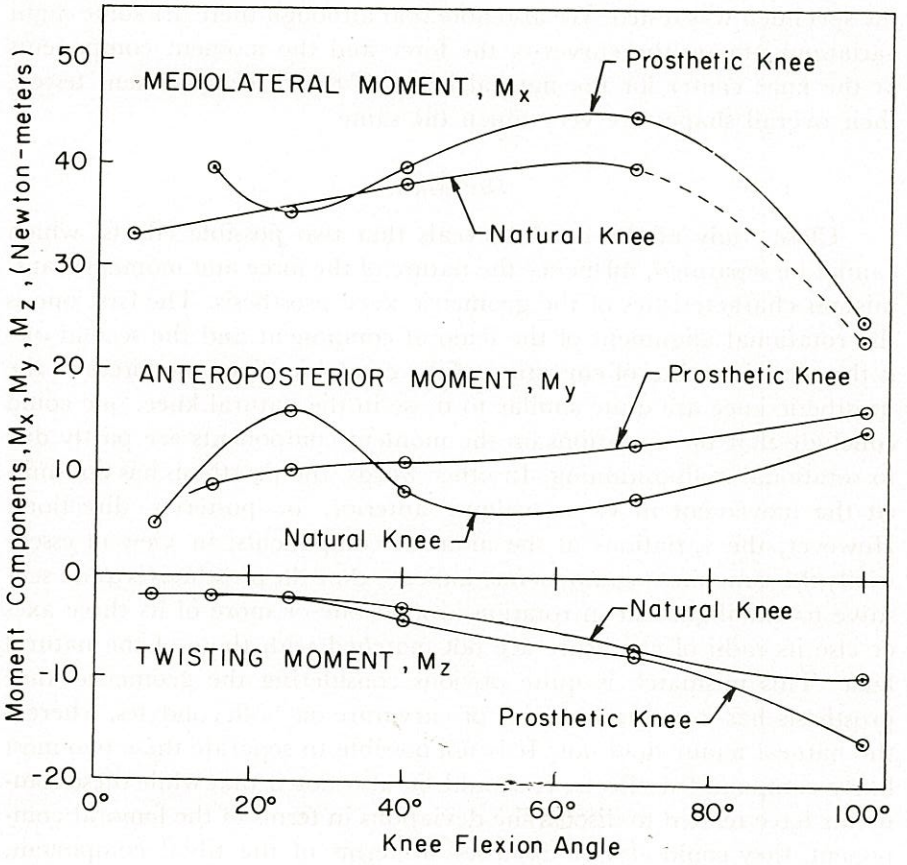

Figure 6

Comparison of moment components at the knee center between the natural and prosthetic knee for various flexion angles with 800 Newtons (81.6 kg) of applied pelvic load. (1 Newtonmeter = 10.2 kg-cm)

vic loads. In this study the knee center was considered to be at 2.5 cm above the contact plane and midway between the condyles. The results are summarized in Figures 5 and 6 where comparison of force and moment components at the knee center between the natural and prosthetic knee is presented for various flexion angles with 800 Newtons of applied pelvic load. Although the force transmission characteristics of the specimen with the natural knee and with a geometric total knee prosthesis are quite similar, there are some differences for the moment components. The strongest differences occur in the behavior of the anteroposterior component, M_y, throughout the flexion range tested, and in the behavior of the mediolateral moment component, M_x, for the initial values of the flexion angle. The variation between the twisting moment components, M_z, is significant at the largest flexion angle for which the specimen was tested. We also note that although there are some slight variations among the curves of the force and the moment components at the knee center for the natural knees of the three specimens tested, their overall shapes are very much the same.

Discussion

Close study of the results reveals that two possible effects, which cannot be separated, influence the nature of the force and moment transmission characteristics of the geometric knee prosthesis. The first one is the rotational alignment of the femoral component and the second one is the relative radius of curvature of the condyles. Since the forces in the prosthetic knee are quite similar to those in the natural knee, one could conclude that the variations on the moment components are partly due to rotational malpositioning. In other words, the prosthesis has not shifted the movement in varus, valgus, anterior, or posterior directions. However, the variations in the moment components, in view of essenttially the same force components, indicate that the prosthesis is quite sensitive to malalignment in rotation around one or more of its three axes or else its radii of curvature are not matched with those of the natural knee. This mismatch is quite obvious considering the geometric knee prosthesis has a constant radius of curvature on both condyles, whereas the natural femur does not. It is not possible to separate these two most likely compounded effects. It should be also noted that while these comments have tended to discuss the deviations in terms of the femoral component, they could also be discussed in terms of the tibial component, or both. The final assessment would be the same.

The results of the present study lead one to conclude that the rotational alignment of the geometric prosthesis seems to be much more

critical than the translational alignment. Furthermore, the dissimilarities of the geometric prosthesis compared to the condyles of the femur are evident in the changes of the knee joint mechanics. In general, it is essential that the force and moment transmission characteristics of any prosthetic device be very similar to the natural joint to assure the lowering of the contact stresses between the prosthesis and bone, thus, reduce substantially any loosening tendencies or microscopic motions at the prosthesis-bone interface which can lead to pain and failure of the implant.

Summary

A critical review of widely used knee arthroplasty devices was presented by classifying them under the titles of hemi-arthroplasty devices, hinged prosthetic devices, duo-compartmental devices and uni-compartmental devices. This classification was followed by delineation of set of design criteria for total knee prosthesis to meet the principal goals of the knee arthroplasty. A report on the comparison of a geometric knee prosthesis with the natural knee was presented and it was concluded that during surgery the rotational alignment of the geometric prosthesis is much more critical than the translational alignment.

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False Positive Liver Scans

(Presentation of Seven Cases)

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The liver scan has been among the physicians' diagnostic tools for many years. It is valuable in the detection of space-occupying lesions larger than 2 cm in diameter. Most investigators define the overall accuracy of the test between 75 to 85 percent.[1, 2] When the liver scan findings correlate with the clinical signs it is a great help[3] for the physician. Conversely, when it is false positive, it puts the physician in a difficult position with respect to the diagnosis and treatment of a patient.

Although false positive rates of 2.5 to 12.5 percent have been reported by some investigators,[4, 5] others appear to have encountered this problem infrequently. This is a report of seven cases with false positive scans as proven by laparotomy or necropsy.

Case Reports

Case 1: E.D., a 35-year-old white female was admitted to the hospital with ascites. The diagnosis of cirrhosis of the liver was established. Au^{198} liver scan showed generalized mottling, an enlarged spleen, increased splenic uptake and a space-occupying lesion over the right lobe (Figure 1). Because of the liver scan findings and high alkaline phosphatase levels, laparotomy was performed. The liver was small and coarsely nodular, but no tumor was found. Operative liver biopsy only showed a postnecrotic cirrhosis.

Case 2: Ş.K., a 33 year-old white male was noted to have had a right upper quadrant pain. Physical examination revealed an enlarged

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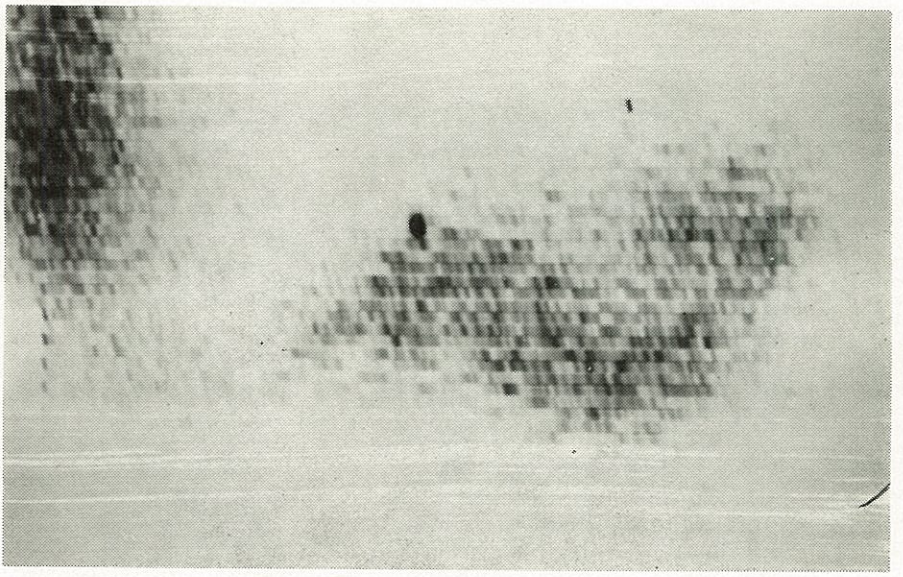

Figure 1

liver. Au^{198} liver scan (Figure 2) showed decreased uptake over the left lobe, inferior and superior aspects of the liver suggestive of metastatic liver disease. The entire gastrointestinal X-ray studies were found normal. A diagnostic laparotomy was performed, liver found to be cirrhotic and the biopsy confirmed cirrhosis of the liver; no tumor was detected, however.

Case 3: K.Ş., a 46-year-old white female, was admitted to the hospital with complaints of right upper quadrant pain. Physical examination revealed an enlarged liver. Au^{198} liver scan showed decreased uptake of the left lobe (Figure 3), lateral scan showed a concave filling defect (Figure 4). Diagnostic laparotomy was performed and showed only an enlarged liver, operative biopsy showed only early cirrhosis.

Case 4: A.A., a 61-year-old white female was admitted to the hospital with complaints of pain over the right upper quadrant. Physical examination revealed an enlarged liver and tenderness over the right guadrant. Gall bladder series showed multiple gallstones. Liver scan showed concave defect in the inferior portion of the right lobe (Figure 5). Cholecystectomy was performed. During the operation multiple gall stones and hydrops of the gall bladder were found. There were adhesions between the gall bladder, colon and omentum.

Case 5: K.Y., a 33-year-old white female admitted to the hospital with large abdominal mass and right upper quadrant pain. Intravenous

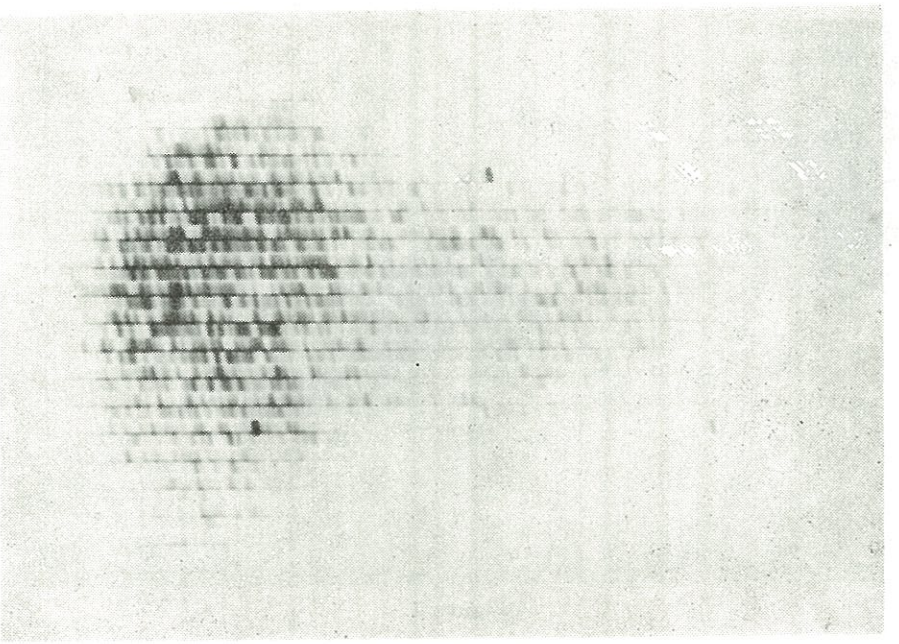

Figure 2

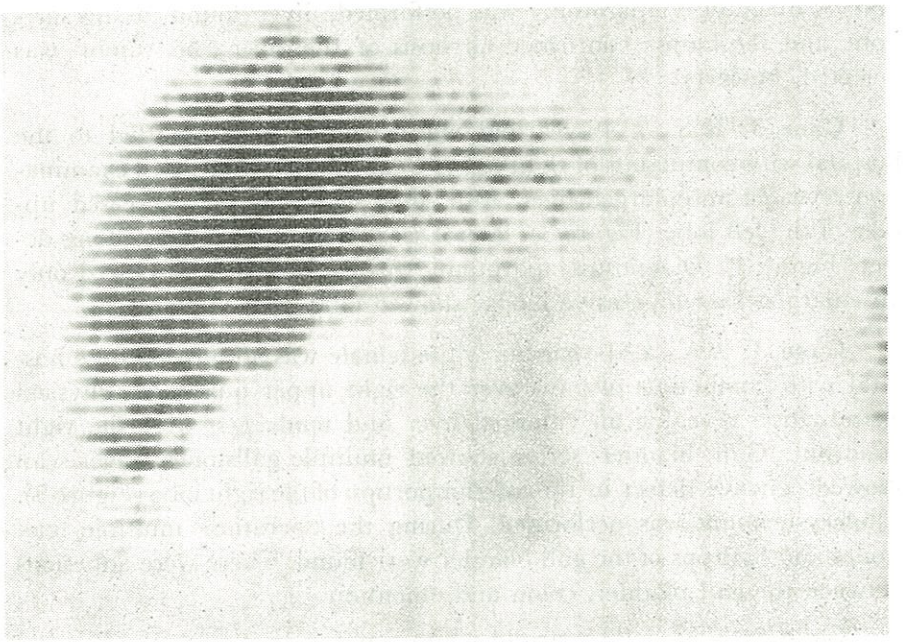

Figure 3

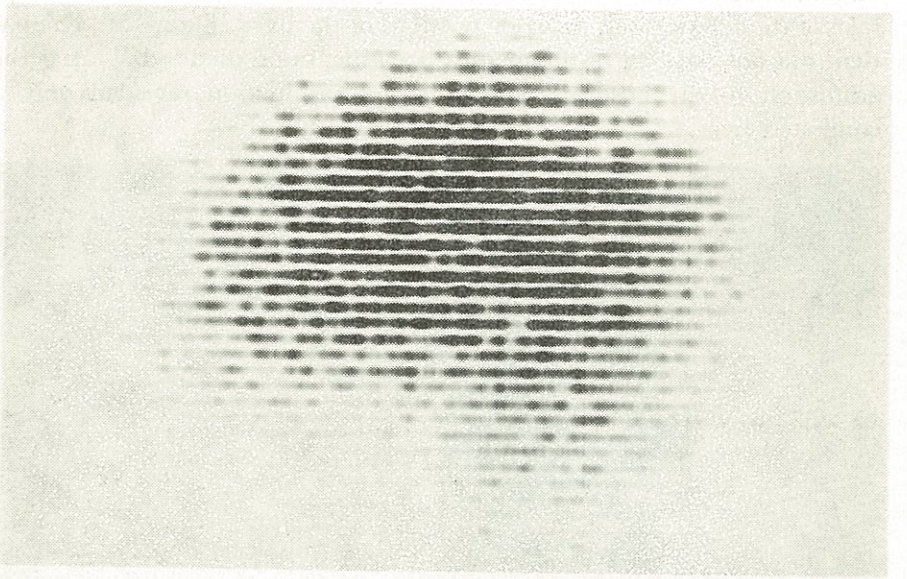

Figure 4

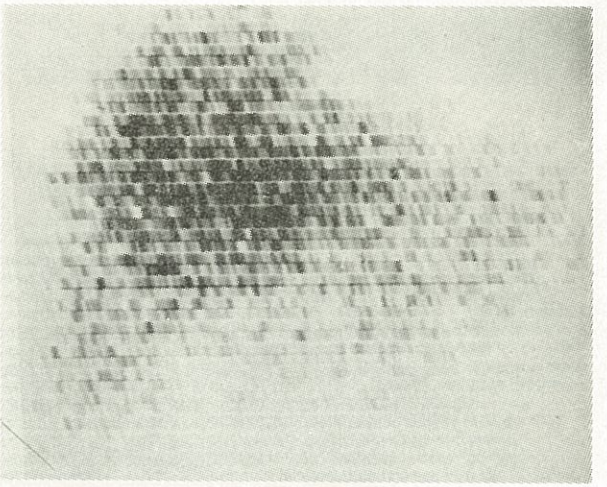

Figure 5

pyelography showed hydronephrosis of the right kidney. Au^{198} liver scan showed a space-occupying lesion between the right and left lobe (Figure 6). At the operation a large hydronephrotic right kidney and a normal liver Were found.

Case 6: K.B., a 68-year-old white male was admitted to the hospital for the fifth time with the diagnosis of chronic lung disease and heart failure. Upon physical examination the liver was found to be enlarg

ed and tender. Au^{198} scan showed an enlarged liver and decreased uptake of the inferior and superior portions of the liver (Figure 7). The patient did not respond to the medical treatment and died 5 days after the admission to the hospital. Post-mortem examination revealed only a congested liver.

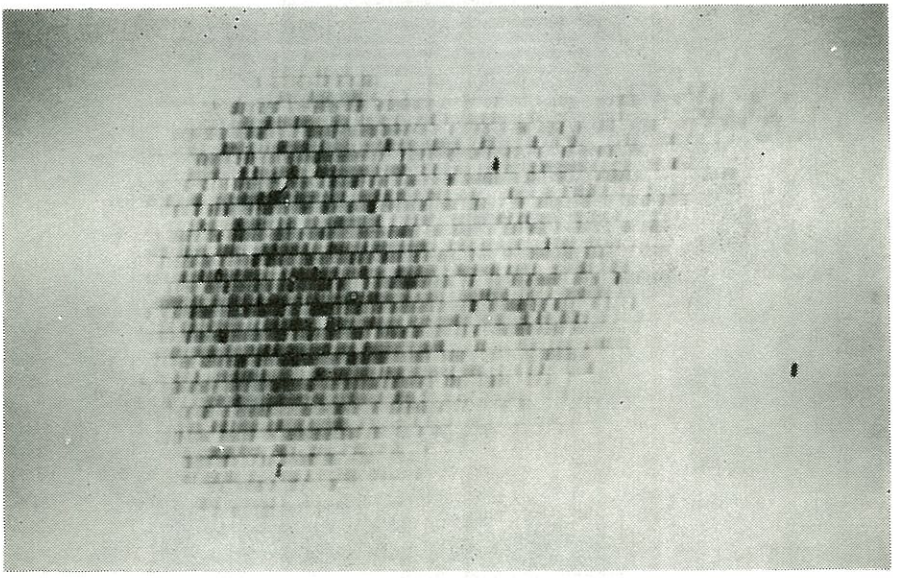

Figure 6

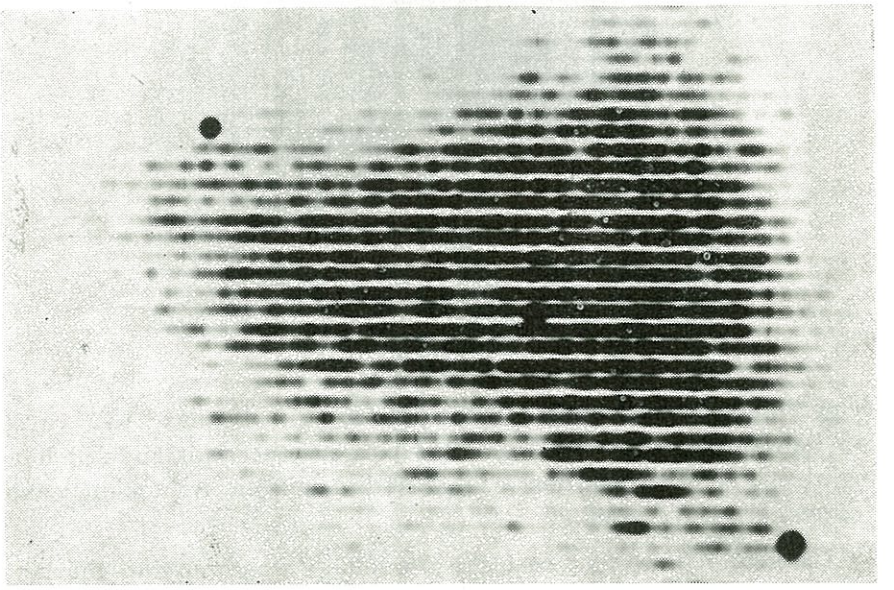

Figure 7

Case 7: H.S., a 62-year-old female was admitted because of an epigastric soft mass 8 x 10 cm in size. All gastrointestinal X-rays were negative. Au^{198} liver scan showed irregular, decreased uptake along the border of the left lobe (Figure 8). Diagnostic laparotomy was performed and a 10 x 10 cm. large pancreatic cyst was discovered. However, the liver was normal.

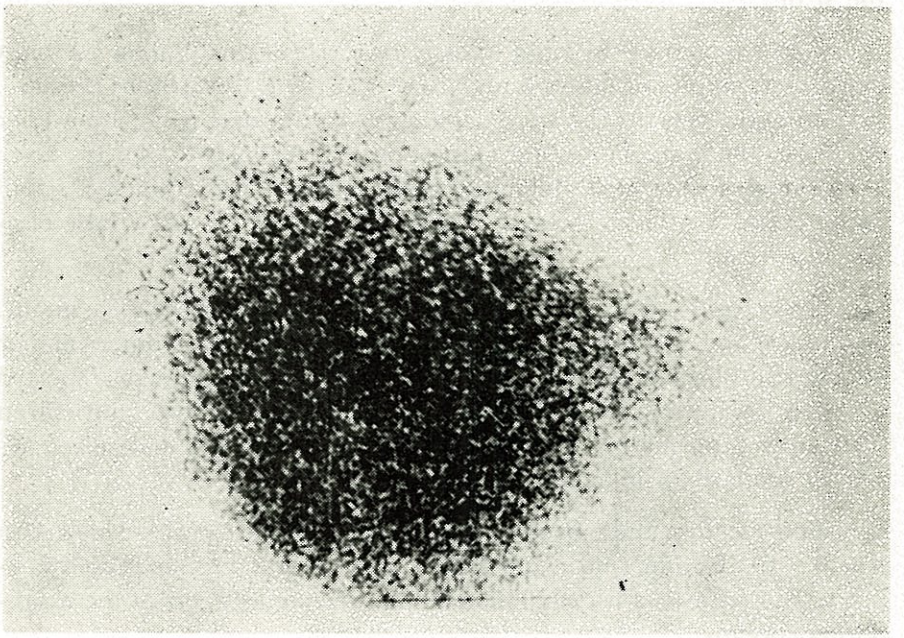

Figure 8

Discussion

When the liver is impringed by disease processes of the neighbouring organs, liver scan is quite confusing since the picture portrayed is frequently indistinguishable from that of intrinsic space-occupying disease of the liver. Enlarged gall-bladder, kidney cyst and tumors, pancreatic lesion, dilated bile ducts and subdiaphragmatic abscess are examples of extra-hepatic disease processes that can simulate intrahepatic space-occupying lesions.[6]

In the first three of our cases the cirrhosis of the liver was found responsible for an abnormal liver scanning. This might be due to decreased perfusion of Kuppfer cells and hepatocytes, fibrosis and finally distortion of the normal anatomy of the liver. Johnson and Sweeney[7] reported false-positive scan in 29 % of their cirrhotic patients with severe portal hypertension. Klion and Rudowsky[8] reported discrete cold areas simu-

lating a hepatic tumor in a patient with alcoholic liver disease. Percutaneous needle biopsies from these areas only showed fatty infiltration of the hepatic paranchyma.

The normal gall bladder is frequently imbedded against the inferior portion of the right lobe. As in our Case 4, when the gall-bladder becomes markedly dilated, a considerably larger defect may be seen on the scan.

Renal lesions of the right kidney have represented one of the most frequent examples of false positive liver scans due to extrinsic pressure. For example hypernephroma of the right kidney may cause a posterior defect on the scan. Polycystic kidneys and perinephritic abscess have been shown to produce similar extrinsic pressure defects on the scan.[6] In our Case 5, a large hydronephrotic right kidney caused a false positive liver scan.

On occasion, large pancreatic cysts may distort the hepatic contour. As in our Case 7, it may cause a decreased uptake of the isotope inferior and lateral portions of the left lobe. Wellish and Holmquest[9] reported a case with traumatic pancreatic cyst. This patient's liver and spleen scan showed an increase in the space between the liver and spleen resulting primarily from compression of the left lobe.

These studies and our cases indicate that the false positive liver scan is not a rare finding and it must be considered prior to laparotomy, especially in patients with cirrhosis and large benign tumors of the neighboring organs.

Summary

False positive liver scans in seven patients were reported. Three out of 7 patients had cirrhosis of the liver. The remaining 4 patients had gall bladder disease, hydronephrosis of the right kidney, chronic congestive heart failure and pancreatic cyst. It is, therefore, evident that the liver scan must be interpreted with caution (especially in patients with cirrhosis) and, when doubt exists, other diagnostic tests should be employed prior to laparotomy.

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Glutathione Content of Human Skin Carcinomas and of Erythrocytes

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Treatment of mice skin with some polycyclic hydrocarbons which induce the skin carcinoma produced a significant rise in the reduced glutathione (GSH) levels of carcinoma tissue.[1] Crabtree found that mercapturate-forming agents and various other compounds known to inhibit sulfhydryl groups acted as anticarcinogens in respect of the induction of skin tumors by polycyclic hydrocarbons.[1] Calcutt and Connors demonstrated that there was a relationship between tumor sensitivity to the alkylating agent and the GSH level of cancer cells.[2] Additionally, a relationship has been found between the protein bound sulfhydryl groups, cellular GSH levels and the average DNA content in certain tumor cell lines.[3,4]

These evidences in the literature suggested that an elevation of cellular GSH levels is an essential prerequisite for tumor formation and the growth rate.

However, an elevation was found in red cell GSH levels in most patients with leukemia and patients with myeloproliferative syndrome which were characterized by unexplained nonleukemic peripheral leukocytosis and hemolytic anemia.[5,6] In this hemolytic anemia no correlation was observed between the red cell GSH content and the reticulocyte count.[5] In addition to that, the age of human erythrocytes plays no significant role in affecting the GSH content of the red cells and the case of intracellular regeneration of GSH from oxidized glutathione (GSSG).[7]

The observation of Eldjarn et al, that neither GSSG nor GSH penetrates the erythrocyte membrane,[8] made it clear that the normal eryth-

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rocyte GSH level must be provided by synthesis of GSH in situ[9] or reduction of GSSG. In the mature erythrocyte lacking the apparatus for division or protein synthesis, the most important role of GSH has to be the prevention of membrane injury against the oxidative stress.[7, 10, 12] Increase in oxidizing power of the surrounding medium of erythrocytes is a factor to maintain appreciable levels of GSH within normal red cells.

This study was designed to obtain the information concerning the GSH level of red cell of patients with localized skin carcinoma and its relation to the GSH concentrations of cancer cells.

Cases and Methods

This study was performed on adult, normal individuals and adult human subjects with skin carcinomas. Intracellular GSH values were investigated in the test materials including the erythrocytes, and the tissue samples were collected from the men suffering merely from skin carcinoma. The control group consisted of fifteen preoperative patients who were in good health and had entered the hospital only for elective surgery, that is hernia and hemorrhoids. Of these patients 12 also donated skin samples for determination of GSH content. All subjects were in normal metabolic state and were taking no drugs or X-ray therapy at the time of the experiments. Three milliliters of fresh blood from each subject was drawn into heparin and it was brought to the cold room within ten minutes after venipuncture. All subsequent manipulations were done immediately. The values for intraerythrocytic GSH concentration were estimated by the method of Beutler et al.[13]

Standard punch biopsies of 4 mm in diameter involving all skin layers were made for sampling. These tissue samples were frozen immediately in tubes containing methanol, cooled in dry ice. Values for tissue GSH content were measured by the DTNB method.[14] Other parts of the same samples were routinely processed and stained with hematoxylin and eosin for microscopic examination.

Results

Test group consisted of 12 patients, there are 10 examples of poorly differentiated to well-differentiated epidermoid carcinoma of the skin and 7 of these occured in men. The remaining 2 had basal cell carcinoma of the skin. The ages at the time of diagnosis ranged from 45 to 75 years with the majority occurring in the 6th and 7th decades. All of these patients without distant metastasis were considered resectable.

The statistical averages of GSH content of the tissues were 0.915 ± 0.073 mg/g wet weight in histologically proven carcinoma cases and

0.126 ± 0.008 mg/g wet weight in control group. The statistical difference, between these two sets of figures was highly significant ($p < 0.001$ / Table I).

TABLE I

COMPARISON OF THE RED CELL GSH CONCENTRATIONS OF THE CONTROL AND TEST GROUPS (in mg/100 ml)

Control Group	Test Group
88.27	87.06
86.54	105.05
88.41	76.03
58.08	114.38
64.65	87.50
73.50	87.12
59.62	135.39
64.44	87.73
82.89	79.21
82.52	107.45
78.59	
73.81	
67.49	
35.45	
96.52	
$\bar{x}=73.38\pm4$	$\bar{x}=96.39\pm5.8$

In the numerous sections of the tests group, there was an obvious correlation between the increasing rate of GSH and the quantity of mitotic figures in carcinoma tissues. No significant difference could be found between the GSH contents of histologically normal skin tissue samples which were obtained from patients with skin carcinoma and of the control group.

The erythrocyte GSH values showed a rather marked range varying from 35.45 to 96.52 mg with an average 73.38 ± 4 mg/100 ml in control group. The intraerythrocytic GSH concentrations of the carcinoma cases showed less variation ranging from 76.03 to 135.39 mg with an average of 96.39 ± 5.8 mg/100 ml. Only one value was below 79.21. The majority of the values were between 79.21 and 107.45 mg. The statistical difference between the intraerythrocytic GSH contents of the test and control groups was significant ($p < 0.01$ Table II).

TABLE I

COMPARISON OF THE GSH CONCENTRATIONS OF THE NORMAL AND CARCINOMA TISSUE SAMPLES
(in mg/g wet weight)

Control Group	Test Group		
GSH Concentration		Site of Tumor	Pathological Diagnosis
0.115	0.655	Lower lip	Epidermoid Carcinoma
0.162	1.030	Right temporal region	" "
0.135	1.278	Left temporal region	" "
0.161	0.914	Lower lip	" "
0.118	1.031	Right temporal region	" "
0.116	0.490	Paranasal area	" "
0.096	1.225	Left maxillary region	" "
0.080	0.640	Lower lip	" "
0.152	1.200	Right scapulo-thoracic	" "
0.160	0.980	Right lateral neck region	" "
0.127	0.731	Nasal region	Basal cell carcinoma
0.100	0.806	Nasal region	" "

$\bar{x} = 0.126 \pm 0.008$ $\bar{x} = 0.915 \pm 0.073$

Discussion

Tumor growth rate plays an important role in respect of the diagnosis and the prognosis of all cancer cases, because the other valuable components of malignancy such as invasiveness, ability to disseminate and to produce metastases or responsiveness to treatment are difficult to quantitate.

The most important biochemical alteration in carcinogenesis is the high glycolytic property of all cancer cells.[15, 16] Isotope investigation of the pentose phosphate pathway suggested that, the rate of oxidation to $^{14}CO_2$ the atom of glucose-1-^{14}C exhibited a positive correlation with tumor growth rate.[17] It was considered that the marked increase in nucleic acid biosynthesis found in malignant cells largely dependent on the high activity of the pentose phosphate pathway which is importantly regulated by the change of the cellular GSH levels.[18]

This study shows that the significant increase in GSH level found in human carcinoma tissue is presumably similar to the elevated level of GSH in experimental carcinogenesis. In addition to that the increasing rate of tissue GSH was comparable with the degree of mitotic activity of human skin cancer tissue which was closely related to the tumor growth rate. In fact the proportion of mitotic figures in histological slides at any given time is, in most cancer cases, a good measure of the rate of growth of the tumor.[19]

Consequently, the level of tissue GSH is an important biochemical parameter which correlate positively with tumor growth rate in human skin carcinoma. However, the significant increase in the red cell GSH level of patient with localized skin carcinoma is not proportional with the GSH level of cancer tissue and the cancer growth rate.

Complete viability of erythrocytes is best maintained under conditions in which the rate of free radicals are generated within the cell or in the surrounding medium of erythrocyte does not exceed the capacity of the GSH-GSSG system to trap and deactivate the radicals.[7] The rate of generation of GSH within the erythrocyte, provides a measure of how fast radical forming agents may be introduced into the circulation without much damage to the red cells.[7, 20] GSH content of red cells has been increased either by reduction of GSSG,[12] or alternatively by synthesis of new GSH.[9] But no mechanism for GSH destruction in the erythrocyte is known.[21] In our previous work, the higher levels of GSH were observed in erythrocytes of human with advanced gastrointestinal tract carcinoma.[22] Also the present study showed that the GSH level of red cells was significantly increased in humans with localized skin

carcinoma. Certainly, this work is suggestive of the skin carcinomas being able to bring about a rise in levels of GSH in red cells.

However, there are several potential points where defect may occur in elucidation of the origin of this reactive material.

Summary

Glutathione has important functions in the process leading to the complete neoplastic transformation of the primary cells in experimental carcinogenesis. This study was made to determine the GSH level of red cell of patients with localized skin carcinoma and its relation to the GSH concentrations of cancer cells. The erythrocytes of patients with cancer showed significantly increased GSH concentrations relative to the controls. Presumably, the significant increase in GSH level found in human carcinoma tissue is similar to the elevated level of GSH in experimental carcinogenesis.

There is no correlation, however, between the GSH content of the red cells obtained from the patients with cancer and that of the cancer cells.

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Radioisotope Scanning in Hepatic Cirrhosis

(An Evaluation of 70 Cases)

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Liver scanning with radioctive isotopes is ferquently used for the study of patients with various forms of liver diseases. Formerly it was considered valuable in the detection of space-occupying lesions, such as hepatic tumors,[1] cysts[2] and abscesses.[3,4] Subsequent studies showed that it is also valuable for the evaluation of patients with diffuse paranchymal liver disease.[5]

The present study was undertaken to evaluate the value of liver scanning in patients with hepatic cirrhosis.

Materials and methods

Seventy patients with cirrhosis of the liver were studied at the Hacettepe University Medical Center. Diagnosis is based on history, physical examination and liver function tests. Liver biopsy was performed only in 37 patients. In the remaining 33 patients liver biopsy could not be performed because of prolonged prothrombin time.

Liver scannings were performed in all patients. An Anger camera with a high resolution, parallel-hole collimator was employed. Three different radioisotopes were used.

Radioactive colloidal gold (Au198) was used in 35 patients. One hour before scanning 100 to 200 m. c. Au198 was injected intravenously

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and liver scans were performed in 3 different positions (anterior, posterior and lateral).

Technetium (Tc99m) was used in 15 patients. 6 milicuries of Tc99m sulphur colloid were administered intravenously. Ten minutes after that anterior, posterior and lateral liver scans were performed.

Indium (In 113m) was used in 20 patients. Six milicuries of (In 113m) were given intravenously and 10 minutes later liver scans were corried out.

Results

The liver scans showed some changes compared to non-cirrhotic normal liver scans. These are as follows:

1. Distortion of normal anatomic shape of liver: Liver size was smaller and the shape resembled a horizontal S (Figure 1).

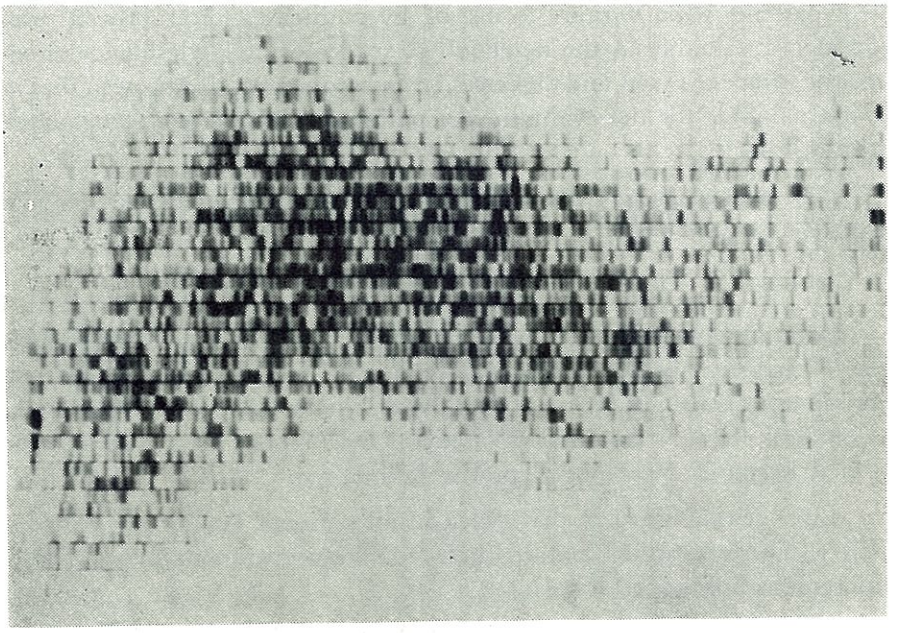

Figure 1

2. Mottling of the liver uptake: Mottling was throughout the entire liver (Figure 2).

3. Enlargement of spleen: Usually observed in patients with portal hypertension.

4. Increased splenic uptake: Splenic uptake was higher than that of the liver (Figure 3).

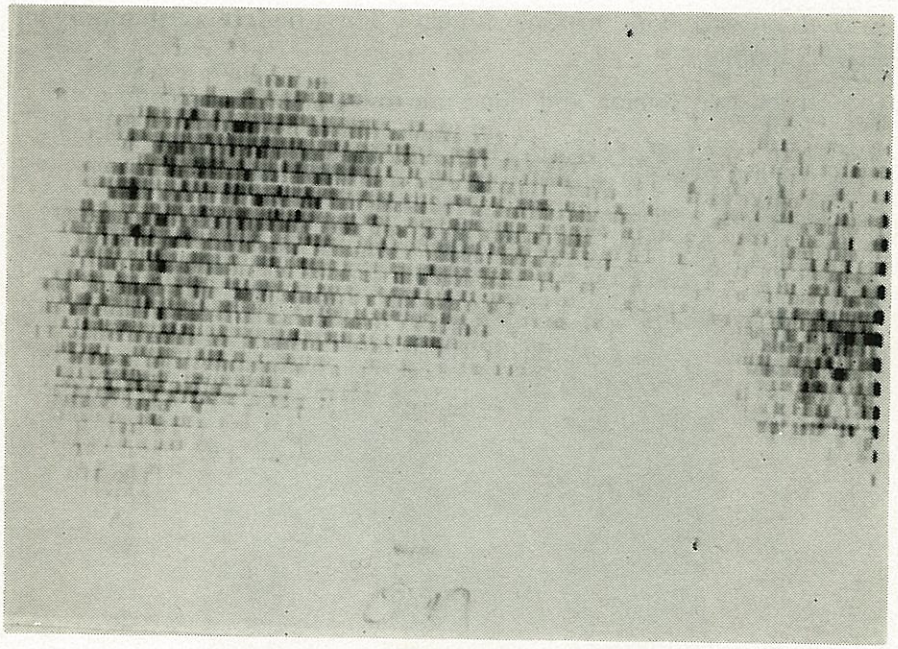

Figure 2

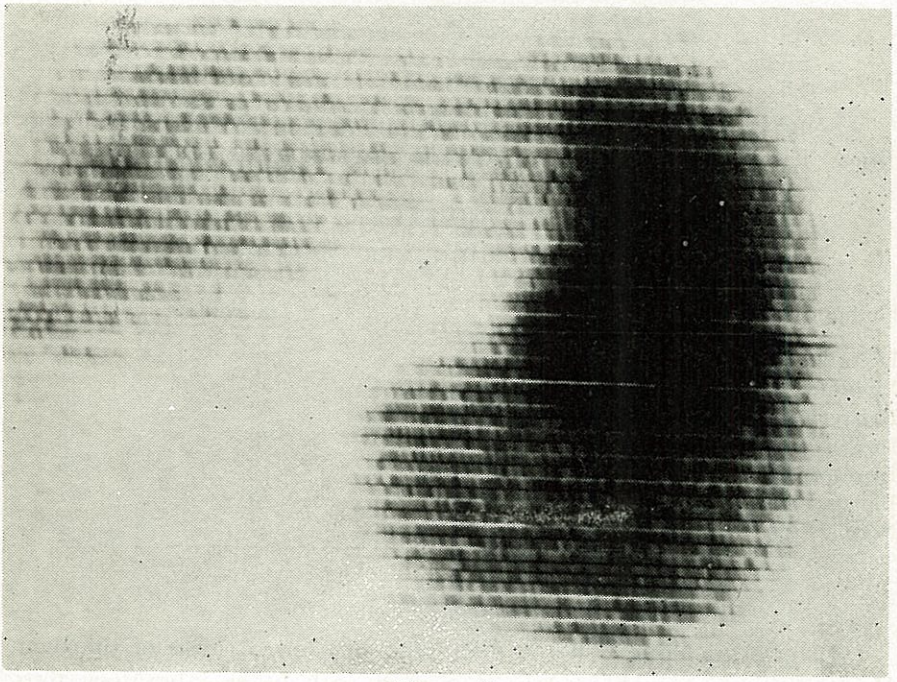

Figure 3

5. Increased bone marrow uptake: This was observed mostly in vertebral bodies.

6. Increased splenic and bone marrow uptake (Figure 4).

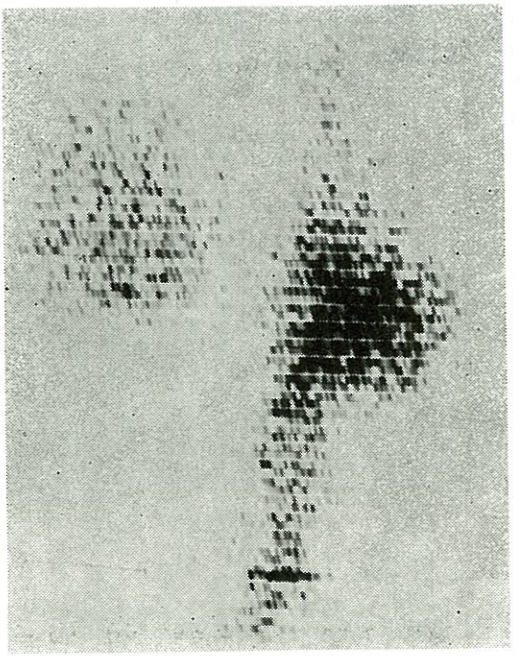

Figure 4

Some of the patients showed combinations of these abnormal findings. Table I shows the liver scan findings of these patients.

TABLE I

CIRRHOSIS OF THE LIVER (70 Patients)

Finding	No. of Patients	%
Distortion of liver shape	48	68.5
Mottling	50	71.4
Enlargement of spleen	30	42.8
Increased splenic uptake	20	28.5
Increased bone marrow uptake	18	25.7
Increased splenic and bone marrow uptake	15	21.4
Normal liver scan	2	2.8

Comment

Liver scan alone is not a diagnostic test for cirrhosis of the liver. However, it is valuable when it is used with other laboratory tests.

The most common finding was mottling of the liver. This is due to decreased liver cell perfusion and the radioisotope excretion as a result of decreased sinusoidal liver blood flow and intrahepatic shunts in these patients.

Severe distortion of the liver in patients with cirrhosis of the liver can be misleading and the scan changes sometimes are indistinguishable from space-occupying lesions. We had one such case (Figure 5). Laparotomy was performed in this patient and only post-necrotic cirrhosis was revealed. Others[6, 7] reported 2.5 % to 12.5 % false positive scans in patients with cirrhosis of the liver.

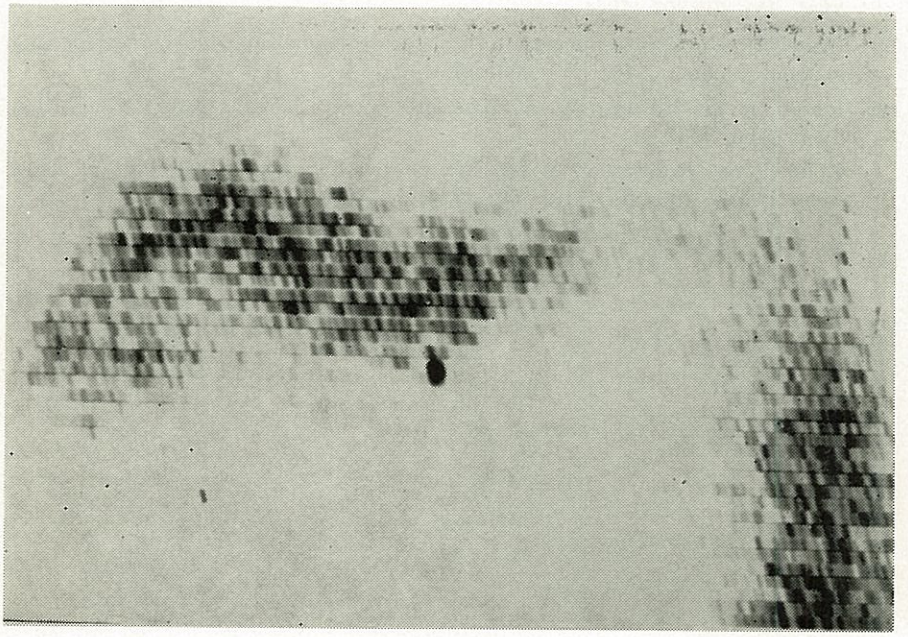

Figure 5

Ten out of these 70 patients showed abnormal liver scan in spite of the normal liver function tests. Since the 68 patients showed abnormal liver scans we thought that liver scans might be a valuable test prior to liver biopsy for the diagnosis of cirrhosis of liver.

Summary

Seventy patient s with cirrhosis of the liver were studied at the Hacettepe University Medical Center. Liver scans were performed on 35 patients with colloidal gold, in 15 patients with Technetium99m and

in 20 patients with Indium 113m. The most prominent finding was mottling of the liver uptake (50 patients). Ten patients showed abnormal liver scan in spite of normal liver function tests.

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The Association of Bronchial Carcinoid and Acromegaly:

A Case Report

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Bronchial adenomata with pluriglandular adenomatosis has been previously reported.[1-3] In some cases acromegalic features and large pituitary fossae coexist with carcinoid adenoma which cannot be attributed to metastases.[4-9] A case of acromegaly combined with bronchial adenoma is presented herein.

Case Report

D.G. (77843), a 32-year-old woman, was admitted to the hospital with complaints of headache and enlargement of her face, hands and feet. She had first noticed these features four years prior.

She had been hospitalized for severe haemoptysis about 15 years ago and was treated with anti-tuberculous drugs under a presumptive diagnosis of tuberculosis. Neither medical nor left artificial pneumothorax controlled her haemoptysis. Pneumothorax led to total collapse of the left lung. The patient continued to undergo medical examination and treatment in various hospitals due to the recurrent complaints.

Physical examination revealed acromegalic features with dullness and diminished breath sounds in the left hemithorax. Roentgenological examination of the chest showed a homogeneous density in the left lung field (Figure 1). X-ray of the skull showed enlargement of the pituitary fosse (Figure 2). Tomography demonstrated complete obstruction of

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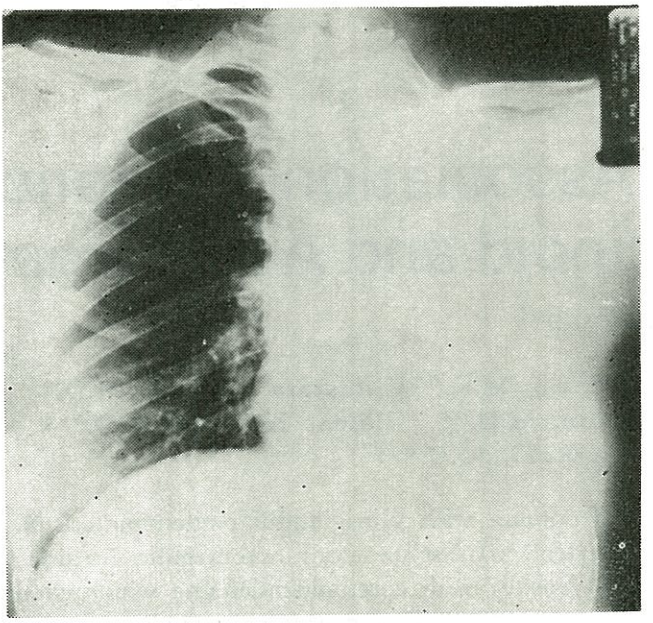

Figure 1

P-A chest demonstrated a homogenous density in the left hemithorax.

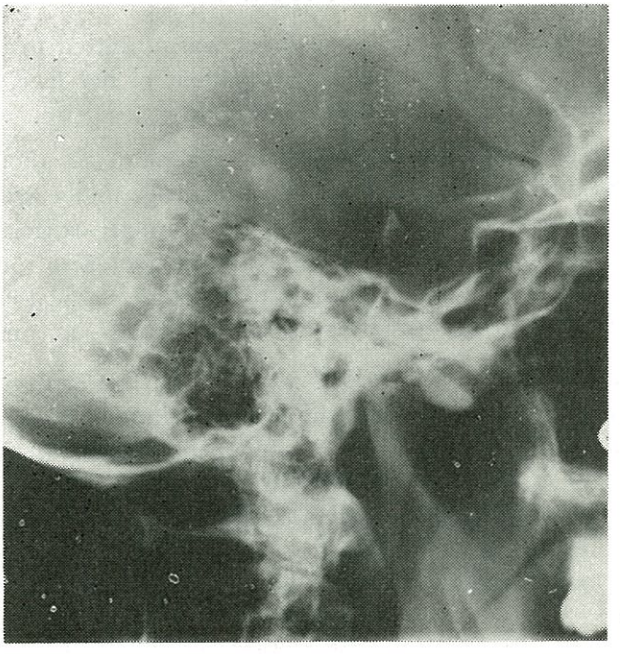

Figure 2

A skull radiograph showing large pituitary fossa.

the left bronchus and calcification in the lung field and pleural surface (Figure 3). At bronchoscopy a pink coloured, easily bleeding tumor was seen in the left main bronchus. Histological examination of brochoscopic biopsy material showed carcinoid type bronchial adenoma. Laboratory investigations including 5-HIAA estimation in the urine were normal except for mild diabetes mellitus which was controlled with 25 units of NPH insulin daily and by diet.

Figure 3

Tomography demonstrated complete obstruction of the left bronchus and paranchimal and pleural calcifications.

Discussion

Bronchial adenomata are uncommon primary tumors of the bronchial tree which occur during the third and fourth decades of life. Their characteristics are gradual enlargement in size and slight tendency to spread to adjacent tissues and rare blood-born metastases. Histologically they can be divided into two distinct groups, carcinoid type and cylindroma. The carcinoid type comprises 85-95 per cent of bronchial adenomas. Bronchial adenomas occur predominantly in women.

Peripherally situated adenomas are often asymptomatic and discovered accidentally. In the majority of the patients, the lesion is endobronchial and symptoms and signs result from bronchial obstruction with recurrent episodes of pulmonary infections. Haemoptysis oc-

curs at least in 50 per cent of the cases, an incidence that reflects the highly vascular structure of these tumors. If the patients have not been subjected to bronchoscopy, they may be erranously treated for suppurative lung diseases or tuberculosis, as was the case in our patient.

These tumors may occasionally produce distinct endocrine features due to their potential hormonal secretory ability. Carcinoid syndrome together with carcinoid type bronchial adenoma is well-known.[9-11] Cushing's syndrome was also found to occur with these tumors.[12-16] Hypoglycemia due to insulin secretion from these tumors has described.[17]

The simultaneous occurrence of bronchial carcinoid and acromegaly was first described by Howes.[5] At the beginning he stated that these two diseases occurred by coincidence. As seven similar cases have been published, this was reconsidered and common pathogenesis appeared to be a better explanation. Secretion of active endocrine substances from these tumors and endocrine-like structures of bronchial carcinoids support this hypothesis.

Summary

A case of carcinoid bronchial adenoma with acromegaly is presented. Endocrine disturbances encountered in these tumors are mentioned. We believe that our case is the ninth one presented in the literature.

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Carbenoxolone in the Treatment of Post-Gastrectomy Bile Gastritis*

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Although post - gastrectomy gastritis is not a very frequent complication of gastric surgery, when it occurs it is a great distress, both for patient and for the physician. Since the previous clinical studies of patients with gastric ulceration have shown that, treatment with sodium carbenoxolone is associated with a significantly increased rate of healing[1, 2] we thought that these patients might have benefit with carbenoxolone treatment.

Material and Methods

Twenty-one patients were studied in Hacettepe University Medical Center. All patients were still symptomatic after the Billroth II operation for the peptic ulcer disease.

Before the initiation of treatment all patients underwent the following studies:

1 – Upper gastrointestinal series
2 – Gastricanalysis
3 – Gastroscopic examination
4 – Gastric biopsy

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Patients with gastric or marginal ulcers are not included in this study.

Upon gastroscopic examination, all patients had a considerable amount of bile reflux into the stomach. Gastric mucosa showed diffuse oedema and hyperemia of mucosal folds. Mucosa was fragile and few patients had superficieal erosions. Gastric biopsies were obtained 2 to 3 cm above the anastomosis. Each patient had 4 biopsies; at 3, 6, 9 and 12 o'clock around the anastomosis. Biopsies showed the following abnormalities:

1 – Mixed cellular infiltration: Mainly lymphocytes and polymorpho nuclear cells.

2 – Oedema and congestion of the mucosa.

3 – Cellular infiltration in the lumen and periphery of the mucosal glands.

Patients were placed on 100 mg carbenoxolone 3 times a day for 2 weeks. Then the dose was decreased to 50 mg 3 times a day. Six weeks after the initiation of therapy gastroscopy and gastric biopsies were repeated.

Results

Upper gastrointestinal series showed only postoperative changes. All had no free acid in gastricanalysis.

Table I shows the clinical, gastroscopic and pathologic changes after the carbenoxolone therapy. Seventeen out of 21 patients had marked clinical improvement, 12 of these showed marked gastroscopic improvement. Fourteen patients showed improvement in the biopsy, 2 of these patients showed only a moderate improvement in their gastroscopic examination. Five patients had moderate gastroscopic improvement and 3 had moderate improvement on pathological examination. Only 3 patients had no clinical improvement and 4 had no improvement either through gastroscopic nor by pathological examination.

TABLE I

POST GASTRECTOMY GASTRITIS
(Treated with Carbonoxalone)
21 Patients

	Clinical symptoms	Gastroscopic Examination	Biopsy
Marked Improvement	17	12	14
Moderate Improvement	1	5	3
No Change	3	4	4

Figure I shows the edema of the mucosa prior to therapy in one patient. Figure 2 shows the oedema, cellular infiltration in the lumen and

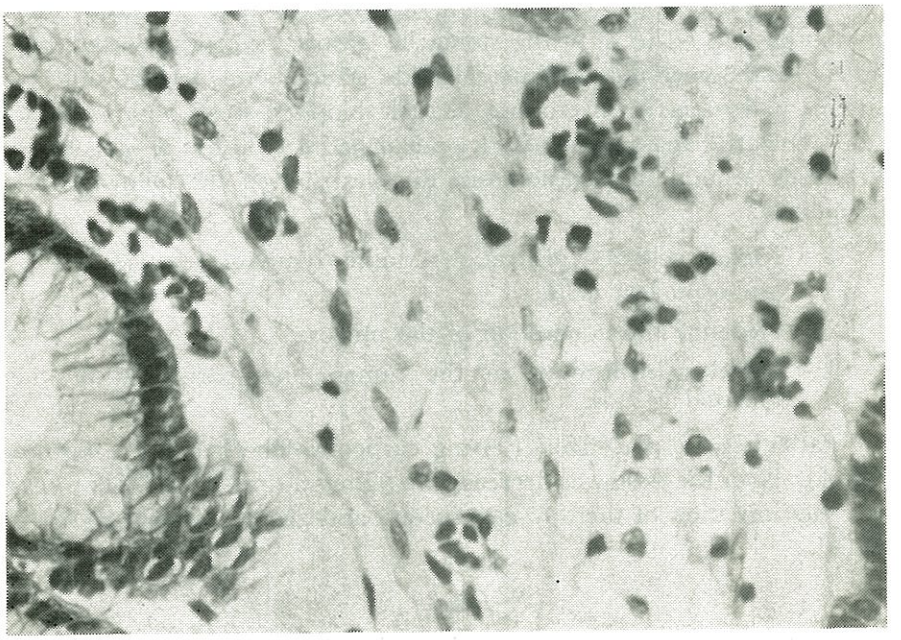

Figure 1

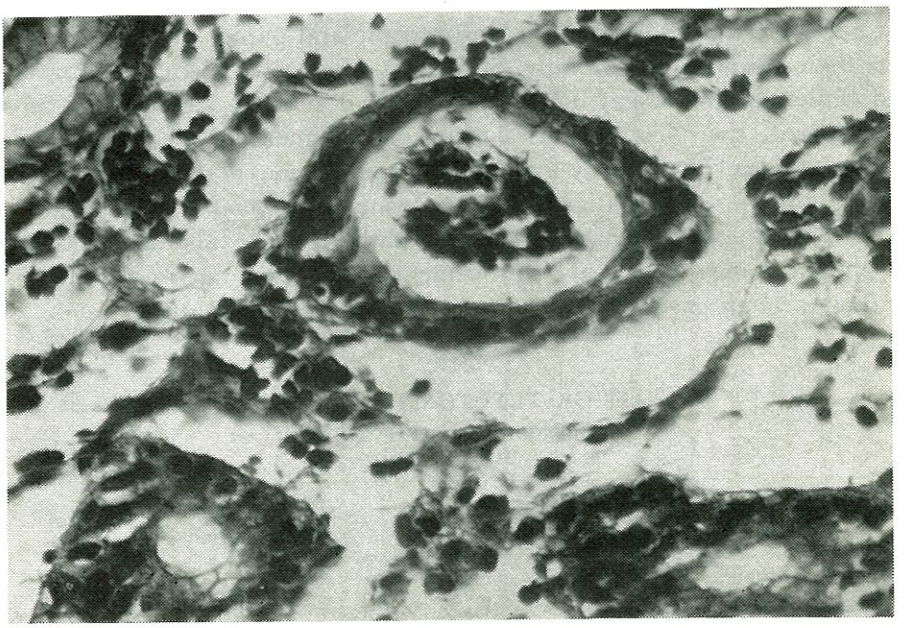

Figure 2

in the tunica propria in another patient. Six weeks after the theapy same patient had marked clinical improvement and his biopsy showed the clear lumen, and decrease of the cellular infiltration, oedema has dissappeared (Figure 3).

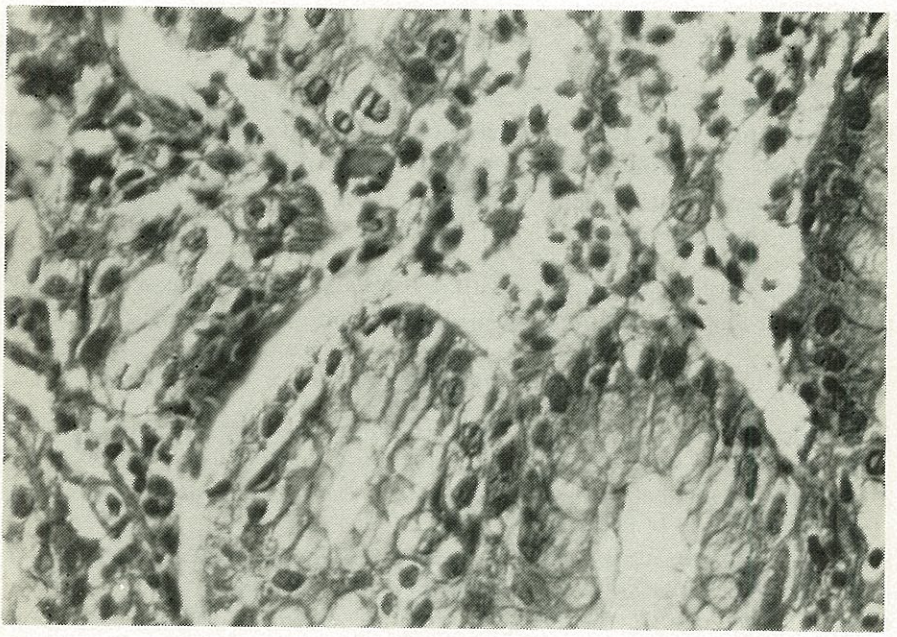

Figure 3

Discussion

Carbenoxolone decreases the turnover rate and increases the lifespan of the mucosal cells. Increase the production, secretion and viscosity of gastric mucus and protects the gastric mucosa against the injurious effect of bile reflux, thus prevents the hydrogen ion back-diffusion.[3-4]

These effects might explain the beneficial effects of carbenoxolone treatment in our patients. However, we must admit that we had to do gastric mucus, and bile analyses before and after the treatment in these patients. This would have given us the better explanation.

In contrast to our results, Gordon's et al studies[5] showed that carbenoxolone does not protect against taurocholate and ethanol induced increases in gastric mucosal permiability in the dog and in man. Also Ivey and Gray assessed the effects of taurocholate instillation prior and following a course of carbenoxolone treatment in human subjects and they

failed to demonstrate a protective effect of carbenoxolone on taurocholate induced disruption of the gastric mucosal barrier.

These studies indicates that, further studies are necessary to clarify the value of carbenoxolone, in the treatment of post-gastrectomy bile gastritis.

Summary

Twenty-one patients who were still symptomatic after the Billroth II operation were placed on carbonoxalone treatment. All patients had gastroscopic examination and biopsy before and after the therapy. The majority of the patients showed marked clinical, gastroscopic and histologic improvement.

In conclusion we might say that the carbonoxalone is a promising medication for the treatment of postgastrectomy bile gastritis.

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