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Lung pathology of atypical pneumonia of unknown origin : with especial consideration of the inclusion body and the hyaline membrane

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THE LUNG PATHOLOGY OF ATYPICAL PNEUMONIA OF UNKNOWN ORIGIN WITH ESPECIAL CONSIDERATION OF THE INCLUSION BODY AND THE HYALINE MEMBRANE.

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I. INTRODUCTION.

During the last few years there has been a great deal of interest in the increasing number of cases of acute respiratory infection associated with atypical pulmonary lesions in which the common pathogenic bacteria do not seem to play an etiologic role. The fact that sulfonamides have no therapeutic effect on this infection has probably been the chief reason for the increase in study of the subject in the last 3-4 years. Many of the reports have come from schools (1-3), colleges (4-8) and army camps (10,11). Hospital personnel, particularly interns and nurses, have also prominently been featured among the reported cases. Although the mortality in these cases has been extremely low the period of disability has been quite long. With the accelerated program of studies now in effect in Universities and with the rapid increase in the size of our armed forces, accompanied as it is by a shorter training period, these cases may constitute a significant problem from the point of view of "time out" from studies or the loss in man-days from training or war industry.

This paper will not deal with work that has been done on the etiology of the disease. Suffice it to say that the board for the control of influenza and

other epidemic diseases in the United States Army uses the designation "primary atypical pneumonia, etiology unknown" (25) for the syndrome which was considered for this paper. They believe that there may be multiple etiologies. T the present time the term most widely used by practitioners is "virus pneumonia". In most cases the only justification for the use of this term is the failure to obtain pathogenic bacteria that can be considered etiologic. Individual authors have justified their use of the term on the basis of suggestive histiologic findings in the pulmonary lesions (16,18,20) or on the results of studies that suggested the presence of filterable agents (13).

A review was made of the literature to determine the predominant pathological findings in the lung in the cases reported at autopsy. Experimental data on the subject was reviewed and an attempt made to find by what pathological processes some of the unusual findings are produced.

II. LUNG PATHOLOGY.

A. Pathological Reports.

Because no single name nor diagnostic criteria have been agreed on for atypical pneumonia I covered all the literature which came under the names commonly applied to a clinical syndrome whose outstanding symptoms are: an incidious onset of prostration, dyspnea and cyanosis; a rasping non-productive cough not associated with any physical findings in the lung in the early stages, but there is an infiltration shown by x-ray. The temperature and blood count are variable, bacteriologic findings are negative and there is no response to the sulfonamides.

Variations of degree and combination of the above symptoms are discussed in the literature under the headings of pneumonitis, virus pneumonia, interstitial pneumonia and atypical pneumonia.

Actually, knowledge of the pathology of atypical pneumonia is limited because of the low mortality of the infection. A small percentage of the literature on the subject contains pathological reports, and many of these are too vague and short to be of any value, so that the descriptions that follow are from the more complete reports.

Bowen (10) reports one death from "acute pneumonitis". At autopsy the lungs showed small areas of pneumonitis. These areas were firm to palpation but not nodular. On section theywere moist with serous and hemorrhagic exudate which was easily squeezed from the consolidated lung. No gross pus was seen. Histological examination showed some cellular infiltration of the smallest bronchioles and of the alveolar walls. Many alveoli were filled with red cells, others with serum. Some alveoli had considerable numbers of mononuclear and epithelial cells but polynuclear cells were not numerous. In other words, sections showed an early exudative inflamatory process.

In 1940 Kneeland and Smetana (14) report one autopsy. The death occurred during an epidemic disease of the respiratory tract of probable virus origin. Grossly the lungs showed fibrous adhesions between the lobes and in places the pleura was covered by a layer of fibrinous exudate. Lung consistency varied: there were many airless patches which projected slightly above the surface and in the intervening spaces was seen moderately emphysematous tissue. On cross section salmon-pink and gray areas of consolidation appeared which varied in size from a few millimeters up to less than a centimeter.

These areas were scattered through the parenchyma and occasionally appeared to be confluent. The cut surface was quite dry. The bronchi contained thick mucopurulent exudate and their mucosa was congested. The bronchial lymph nodes were moderately enlarged and soft. Microscopically the sections showed a varied picture, apparently representing different stages of the same process. The earliest stage was characterized by the presence of a hemorrhagic exudate, composed of erythrocytes, blood plasma and a few mononuclear wandering cells within the alveoli. The septa were of normal width and their capillaries appeared rather bloodless. Occasional septa showed mild infiltrations consisting of mononuclear wandering cells and polymorphonuclear leucocytes. Some of the alveoli showed large mononuclear cells arranged about the periphery. All stages, from the appearance of single cells and small rows, to a continuous lining of the alveoli, could be followed. A somewhat later stage showed alveoli which contained poorly preserved erythrocytes with a tendency to clumping and hyalinization. There was a marked tendency to organization by fibroblasts and endothelial cells which penetrated into the exudate, incorporating it into

the septa. A moderate number of polymorphonuclears were present in the exudate and in the alveolar septa. Again large mononuclear cells were seen to line many of the alveoli, forming continuous rows. These rows appeared frequently detached from the alveolar walls. Many of the alveolar spaces were solidly filled with mononuclear wandering cells containing small fat droplets and hemosiderin pigment granules. An occasional hyaline membrane was present. With progressing organization the alveolar spaces showed a marked tendency to become smaller, while the septa increased in thickness. The peri-alveolar capillaries were never very prominent. The medium sized branches of the pulmonary artery showed extensive, usually eccentric necrosis of their walls which were infiltrated with polymerphonuclears, mononuclear wandering cells and eosinophils.

The most advanced stage of the consolidation was characterized by extreme thickening of the alveolar septa. The alveolar spaces were quite small, some were empty but more frequently they contained debris, fibrin, lymphocytes, large mononuclear wandering cells holding fat drop-

lets and hemosiderin pigment granuoles or both. No inclusion bodies were seen in any of these sections. The septa showed extreme thickening which was due to fibrous tissue and infiltrations consisting mainly of nononuclear wandering cells. The peri-alveolar capillaries appeared bloodless and were often buried in the center of the septa. These were seen, however, in an increased number of small arteries, arterioles and veins whose walls appeared quite thick. The medium sized and larger branches of the pulmonary artery again showed partial or complete necrosis of the media and the intima, sometimes accompanied by partial thrombosis of the lumina. The mucosa of the larger bronchi and of the trachea was densely infiltrated by polymorphonuclears and mononuclear wandering cells. Their epithelial lining cells were quite prominent and frequently projected into the lumina in the form of small papillae. Many mucous forming cells were present among the lining cells. Some of the lumina contained mucopurulent exudate and debris. Sections of bronchial lymph nodes showed acute lymphadenitis.

Longcope (15) reports two deaths from a series of thirty two cases of "Brenchopneumonia of unknown etiol.";

One death occurred in a thirty eight year old woman. At autopsy the lungs were heavy and boggy. Pleural surgaces everywhere were smoothe and glistening. Blood vessels show nothing remarkable. Bronchi show everywhere greatly reddened mucosa and are filled with frothy pink material. There were areas in left upper, lower and rithat lower lobes which felt almost completely consolidated, but on section these appear to be rather filled with fluid than consolidated. Every lobe showed a great amount of pinkish fluid in the alveoli, perhaps somewhat more in the lower than in the upper lobes. Here and there were small patches of slightly raized dark areas,about 2-4 cm. in diameter, which were probably small patches of consolidation. The hilar lymph nodes showed nothing remarkable. Microscopic examination demonstrated about the same thing all over the lungs, except moreso in the lower lobes. The bronchi were everywhere filled with a very thick exudate consisting of about half polys and half round cells. Their walls were infiltrated with round cells and occasional polymorphs and many

of the alveoli were solidly filled with round cells. Sections appeared very intense and in some places there was difficulty in making out the bronchi. The striking thing was the almost complete absence of polymorphs except in the bronchi. Bacteriological stains failed to reveal any organisms in either the bronchi or the alveoli. There was one other rather striking observation, and that was the change which was noted only in the larger bronchi and which was a squamous metaplasia of the bronchial epithelium which in places could be seen to be pushing up the normal epithelium and replacing it.

The other case reported by Longcope was that of a 40 year old man (The patient had also had rheumatic fever). In the upper part of the left lower lobe was a wedge-shaped area of consolidation, measuring 5 cm. in greatest diameter. The pleural surgace here was black and covered here and there with a fine fibrinous exudate. On section this part of the lung was dark red throughout and hemorrhagic. The bronchi were patent but considerably reddened. Some were filled with a tenacious mucoid material while others contained purulent material. On section a few small areas of consolidation were made out in the lower lobe; the

upper lobe was soft throughout. Microscopic examination showed some organization of old exudate in the alveoli. In others there was hemorrhage. Alveolar walls were intact. Some of the smaller radicles of the pulmonary artery showed necrosis and cellular infiltration of the wall. In other regions there was fresher exudate in the peribronchial alveoli which was filled with polys. A few gram-positive cocci were stained here. Sections from the left upper lobe showed fluid in the alveoli and also macrophages containing altered blood pigment.

Out of 244 cases of "atypical pneumonia" reported by Dingle (46) at Camp Claiborne, La. there was one fatality. Results of gross pathological examination of this one case were chiefly firm, dark, hemorrhagic consolidation of the apical half of the upper lobe of the left lung, of the hilar portion of the right lung and of the lower lobe of the right lung; plugging of the small bronchi; slight enlargement of the spleen, and enlargement of the mediastinal lymph nodes. The amount of pleural fluid was not abnormal. Microscopically, the involved areas of the lungs showed congestion and infiltration of the alveolar walls with mono-

cytes, lymphocytes, plasma cells and occasional neutrophils. A similar reaction was present in the peribronchial interstitial tissues. The alveoli contained many large actively phagocytic monocytes, a moderate number of eosinophils, a scant number of neutrophils, fibrin, edema fluid and red blood cells. The walls of the bronchi showed an acute inflamatory process with extensive ulceration of the bronchial mucosa; the lumens of the bronchi contained an exudate composed chiefly of neutrophils. Neither bacteria nor cellular inclusions were observed. The findings were thus those of hemorrhagic interstitial bronchopneumonia, acute bronchitis, and mesenteric lymphadenitis.

Dingle did a bronchoscopic examination of 10 patients with atypical pneumonia and 1 pneumococcic pneumonia. There was an acute inflamatory reaction of the mucosa of the involved lobes, with considerable congestion. In some patients the mucosa bled easily. The secretion was mucoid to mucopurulent. The involved bronchus of the patient with pneumococcic pneumonia presented a moderate degree of inflamation, but the reaction was not so acute as in the patients with atypical pneumonia, and no bleeding occurred when the mucosa was wiped with a swab. Uninvolved bronchi and lobes were essentially normal.

Pediatritions seem to report a response more acute in character in which bronchial ulceration and hemorrhage are prominent changes.

Adams, et al summarize their findings in two epidemics of "virus pneumonitis": Prominent pathological features were proliferation and sloughing of bronchial epithelium. The exudate in the bronchial lumen was primarily epithelial in character, and the principle parenchyma was mononuclear. Atelectasis, edema and hemorrhage were noted. Cytoplasmic inclusion bodies were found in the diseased epithelial structures of the lung. These bodies varied from 3-6 microns in diameter, stained acidophilic with the hematoxylin and eosin and also with the Giemsa stain, were often surrounded by a clear halo and in a few instances contained vacuoles.

Goodpasture, et al summarize 5 cases of "virus pneumonia" which occurred as a fatal secondary infection in infants suffering from epidemic infections. They describe this type of pneumonia as a secondary infection especially common in measles and whooping cough.

Grossly in each case the trachea and bronchi showed hyperemia, and their mucosas were dulled and thin, suggesting ulceration in places. Consider-

able stringy mucoid material was present within the lumens. The pleural cavities contained no excess of fluid, and the surgaces were smooth and glistening. When the lungs in each case were sectioned, scattered areas of consolidation were observed. A particular feature of the consolidated areas was their relation to bronchi and bronchioles. In the center of each of these areas was a softened, opaque spot or line, variable in size and consisting of pus surrounded by a thin zone of pneumonic consolidation which often was red itself or was encircled by a zone of hemorrhage in their cases. The central areas had the gross appearance of small bronchiectatic abscesses or of bronchi and bronchioles filled with exudate. Microscopically, each case the surface of the trachea and the larger bronchi was completely denuded of ciliated epithelium and was in part ulcerated. The remaining surface was covered by fibrin. There were scattered areas of necrosis in the mucous glands, involving one or more lobules. This was one of the most characteristic features of the process.

Just beneath the basement membrane of the mucosa there was an exudate of polys, especially abundant at areas of ulceration; in other places it was admixed

with mononuclear cells of various sorts. Polys were present in and on the epithelial layer.

The smaller bronchi and bronchioles frequently showed complete destruction of epithelium. The lumens were filled with cellular exudate and necrotic debris, and frequently bacteria could be demonstrated in them. The lumens were distended with leukocytes and were surrounded by inflamatory exudate. Extending beyond and about the bronchioles were patches of pneumonitis involving alveoli. The exudate varied in composition. At times it was predominately hemorrhagic and in places was fibrinous and cellular. Polys and monocytes were the chief cellular elements, although other mononuclear cells were present. In the neighborhood of alveolar ducts the alveolar and ductular exudate was extremely compact, and there was much fragmentation of nuclei. This gave a rather characteristic appearance which suggested the early lesions of tularemia. Alveolar walls were involved in the necrotizing process, which extended outward from the bronchioles.

Gedgoud (61) reports findings in a six months old white female, following death from "virus pneumonitis". The lungs were markedly distended except for an area of consolidation involving three-fourths of the left

upper lobe. All of the bronchi throughout both lungs contained purulent material, occluding their lumina. Microscopic sections of lung tissue showed proliferation and sloughing of the epithelium plus mononuclear cells choking the bronchioles, which were surrounded by a peribronchiolitis. Under oil immersion, numerous inclusion bodies were easily demonstrated in bronchial epithelium. One inclusion body appeared to be intranuclear but all of the remainder were in the cytoplasm.

By way of summary there are four main points to keep in mind:

1. Early in the alveoli there is a hemorrhagic exudate composed of erythrocytes, blood plasma and mononuclears whose number depends on the stage of the process. Later alveoli show poorly preserved erythrocytes with a tendency toward clumping and a mild invasion of polymorphs and a few fibroblasts. Occasionally a hyaline membrane is present. It was pointed out several years ago (91) that the presence and the degree of hyalin present in influenza pneumonia depends on the amount of dyspnea experienced by the patient.

2. The alveolar septa contain a moderate amount of polymorphs and mononuclears and as the process continues these walls thicken due to the organization by fibroblasts and endothelial cells and the infiltration of mononuclears. This thickening causes a decrease in the size of the alveoli.

3. Smaller blood vessels usually show thickened walls whereas larger vessels show partial or complete necrosis of the media and intima and sometimes a partial thrombosis of the lumina.

4. The larger bronchi show infiltrations similar to those found in the alveolar septa except there are

usually more polymorphs present. Also, the epithelial lining cells are quite prominent and frequently project into the lumina in the form of small papilla. Apparently it is in the more severe cases that this regeneration is replaced by ulceration.

B. Experimental Data.

The experimental phase of atypical pneumonia has not yielded much helpful information. The is partially due to the fact that recovery of the pathological agent is an exceedingly difficult procedure, and secondly, atypical pneumonia has not been recognized long enough as a separate clinical entity to have aroused the interest of very many investigators.

In 1938 Weir and Horsfall (41) successfully produced a pneumonitis in the wild mongoose from the throat washings of patients that were diagnosed clinically to have atypical pneumonia.

Nutrient broth, or a broth-saline mixture, was used to obtain washings of the throats of patients during the first few days of the disease. These washings in some instances were mixed with equal parts of glycerin for transportation; others were frozen solid with carbon dioxide and transported in the frozen state. The glycerinated specimens were stored at 4° C., while the frozen washings were kept in a low temperature storage cabinet at -76° C, The type of storage state used seemed to make no difference in the results obtained. Filtered and non-filtered specimens gave the same results.

Among the animals inoculated intranasally with throat washings from a number of clinically typical cases were ferrets, mice, guinea pigs, rabbits, monkeys, oppossums, skunks, woodchucks, voles, deer mice, and Syrian hamsters. Serial lung passages by intranasal inoculation were also made in ferrets and mice. In no instance did the inoculated animals develop evidence of infection, and at autopsy no lesions were found in their respiratory tracts.

Since the ferret is succeptible to infection by influenza A virus, it was decided to attempt to produce virus pneumonia in another animal of this family found in Jamaica- namely the Mongoose.

2 cc. of throat washings were inoculated intranasally in mongooses anesthetized with ether. At the end of a 10-12 day period the animals were anesthetized, bled by cardiac puncture, and killed. Their lungs were removed aseptically and examined. Primary infections were produced and the virus was maintained by serial passage. Moreover, when filtered throat washings were inoculated directly onto the chorioallantoic membrane of the developing chich embryo, the virus was found to be present in the embryos through at least 30 serial passages.

Although there were slight differences observed

in reactions of the animals, two distinct types of lesions were noted. These consisted of circumscribed areas of consolidation and generalized pulmonary hyperemia. The consolidations were plum-colored and of a gelatinous consistency. They varied in size from small areas 1mm. or more in diameter to large lesions filling an entire lobe. The distribution of the consolidations varied from single large lesions in one lobe to multiple small lesions in a number of lobes. Pulmonary hyperemia was usually generalized and was found regardless of the presence or absence of consolidations. Hyperemic lungs were of a deep red color in striking contrast to normal mongoose lungs which were light gray in color with a pinkish tinge. Histologically, the lungs showed an extensive edema which filled and distended the alveolar spaces and caused thickening of the alveolar walls. There was a sparse cellular exudate composed almost entirely of mononuclear cells. The bronchial epithelium was well preserved and the bronchial lumina contained small amounts of protein-rich edema fluid. Neither perivascular nor peribronchial cellular infiltrations were seen.

In 1933 Mc Cordock and Muckenfus (38) produced

a vaccine virus pneumonia rabbits. Although the strain of virus employed was not obtained from patients having had atypical pneumonia (the neurovirus of Levaditi was used) there are several points that are important for our consideration. The results obtained represent use of over 100 animals.

The virus was prepared by diluting with Locke's solution an emulsified rabbit testicle 4 days after intratecticular inoculation with the neurovirus of Levaditi. In experiments in which virus alone was used 1 to 2 cc. of a 1:10 dilution were injected. When bacteria were also injected a 1:10 solution proved too strong, so that a 1:100 dilution was employedin the latter experiments.

Twenty-four hours after injection of virus (without bacteria), there is a marked tracheitis and bronchitis with a red, swollen mucosa. The lungs showed elevated patches of translucent gelatinous consolidation, some of which became hemorrhagic after the second day, producing firm, dark red to purple-colored areas. About the third or fourth day, on a cut surface of the lung in an involved area the bronchi are prominent due to thickening of the walls. In an occasional animal this thickening and infiltration extended beyond the bronchioles to produce small yel-

lowish peribronchiolar nodules resembling tubercles, which were very conspicuous where the lung tissue forming the background was hemorrhagic.

Microscopic study shows the areas of gelatinous consolidation, the earliest gross lesion, to consist of groups of alveoli filled with coagulated edematous fluid and varying amounts of fibrin. At this stage the alveolar walls showed no structural change, although their capillaries were at times congested. Mononuclear cells were at times found in the alveoli in small numbers. In later stages this lesion resolved itself in two different ways. In the most acute reactions the alveolar epithelium degenerated or desquamated. Damage of the capillaries caused hemorrhage into the alveoli. As the lesion develops the entire alveolar wall, including the capillary, degenerates and in this way large irregular areas of lung tissue, although retaining their structural outline, became completely necrotic, resembling the center of an infarct. If the animal survived, great numbers of polymorphonuclear leukocytes invaded the necrotic tissue. Mc Cordock and Muckenfus suggest that this lesion be called "hemorrhagic virus pneumonia". Instead of this hemorrhagic and necrotizing process a proliferative cellular lesion may develop. When this

takes place the bronchioles and the alveolar walls become thickened by an infiltration with mononuclear cells. The infiltration is most marked in the alveoli close to a bronchiole or in the neighborhood of a blood vessel, the adventitia of which is thickened and infiltrated. The cells of the alveolar epithelium enlarge and the great frequency of mitotic figures clearly indicates their active multiplication. These large mononuclear cells become more numerous in the alveoli and in some they displace other elements. It is proposed by Mc Cordock and Muckenfuss that this proliferative reaction be termed "interstitial virus pneumonia". These two lesions occurred both separately and together. Cytoplasmic inclusions were found in about 10 per cent of non-immune animals.

The two types of lesions observed were not regarded as different stages of the same process but rather as quantitative reactions to different concentrations of virus. A large quantity of strong virus injected into the lungs of a normal animal tended to always produce hemorrhagic virus pneumonia with extensive necrosis, a minimal cellular proliferation and early death. A small quantity of it, or a more dilute virus, called forth the prolifer-

ative cellular lesions of interstitial virus pneumonia, which in not immediately fatal. A moderate amount of virus introduced into the lungs of a vaccine-immune animal, or the injection of virus mixed with immune serum into a normal animal, resulted in a proliferative lesion, if there was any reaction at all.

All the lesions so far described occurred in the absence of bacteria. Repeated cultures of lung tissue remained sterile, and bacteria were never found in the many sections of the most severe lesions stained by various methods for the demonstration of bacteria in the tissues.

Mc Cordock and Muckenfus also observed the effect of superimposed bacterial infection on the virus pneumonia. Three types of bacteria were used. One was hemolytic streptococcus isolated from a spontaneous infection in a rabbit. Another was a stapholcoccus cultured from the lung of a child with postpertussis interstitial bronchopneumonia, and the third organism was a stock strain of B. pertussis. These organisms were grown on blood agar slants from which thin suspensions were prepared in salt solution.

The only reaction noticed after injection of

large quantities of B. pertussis was a mild inflammation of the tracheal and bronchial mucosa.

The lesions or complications superimposed on virus pneumonia by the simultaneous or subsequent introduction of Staph and Strep were as follows: (a) acute bronchitis and lobular or bronchopneumonia; (b) abscess formation; (c) pleurisy and empyema; and (d) bronchiectasis, squamous cell "metaplasia" of bronchial epithelium and organizing pneumonia.

The squamous cell metaplasia is of especial interest because, it will be recalled, that this finding was often mentioned in the pathological reports of human material.

In 1938 Francis and Magill (90) while making an extensive study of epidemic influenza isolated a virus from throat washings which was immunologically diffenent from that producing influenza and yet like it in many respects. The viruswas pathogenic for mice, ferrets, and monkeys of both M. rhesus and M. cynomolgos species.

In the ferret the involved lobes are plum-colored, firm and distended with edema fluid which flows freely from the cut surface of the bronchi. At times a clear albuminous fluid is present in the pleurat cavity. Microscopic examination reveals

edema of the bronchial walls but little or desquamation of the bronchial epithelium; in fact it appears somewhat hyperplastic. Exudate may be formed in the lumen of the bronchus. The vascular endothelium appears swollen and unusually prominent but hyperemia is not an outstanding feature. The alveolar walls are edematous and densely infiltrated with large mononuclear cells containing large pale nuclei. These cells containing the pale nuclei are seen to almost form a lining of the alveolar spaces and the appearance of the lung in places approaches the andenomatous. The alveolar spaces are distended and contain a moderate cellular exudate consisting primarily of large pale-staining mononuclear cells. Polymorphonuclear leukocytes are not prominent. Fibrin is usually not observed, and edema fluid, despite the large amount which seeps from the cut lung, is re realitively scanty. In general, however, the lung presents a picture of a more proliferative type than that observed in infection with the virus of influenza.

The pathological lesions in the lung of infected mice by intranasal inoculation is similar to that described in ferrets except there is

greater hyperemia and edema.

In mice which develop paralysis following intracerebral or intraperitoneal inoculation there is a meningeal and choroidal reaction of a mixed mononuclear and polynuclear type, the proportion of the latter cells being apparently related somewhat to the acuteness of the disease.

Subcutaneous inoculation of mice, monkeys, ferrets, rabbits, and guinea pigs causes local granulomatous induration of the skin with enlargement of the regional lymph nodes.

In 1941 Eaton, Beck and Pearson (42) reported the isolation of a virus from the lungs of two fatal cases and from the sputum of two non-fatal cases of atypical bronchopneumonia. Each ease was isolated separately and all of them found to be of the same strain. This strain was antigenically related to, but not identical with, the strain of virus isolated by Francis and Magill (90) and named by them the virus of meninge-pneumonitis. Both of these strains are also antigenically related to psittacosis virus from parrots.

The virus thus isolated was found to have a relatively high virulence for mice by intranasal or intracerebral inoculation, but does not kill after in-

traperitoneal inoculation. Its virulence for Java ricebirds is low and it fails to produce a carrier state in mice and birds.

The appearance of lung lesions in mice depended upon the stage of the disease and the amount of virus inoculated. High dilutions did not produce a uniformly fatal disease and small round grey foci were seen. With larger doses the foci became confluent and solid or patchy greyish pink lesions were produced. In mice dying acutely after intranasal inoculation of ten per cent suspensions, the lungs were completely consolidated, deep red, and very edematous. There was a small amount of sticky pleural exudate. The various lesions observed were not dissimilar from those seen in mice inoculated intranasally with parrot strains of psittacosis virus (42).

Microscopic pathology was identical to that described by Francis and Magill (90).

Subcutaneous inoculation of mice showed no definite lesions except a slight enlargement of the regional nodes.

The apparent lack of thoroughness in review of some of the above material is due to the practice of brevity and generalizations made by many of the authors.

C. The Inclusion Body.

The discovery of inclusion bodies by Adams and Goodpasture (16, 18) is of interest because these bodies have been described in connection with most virus diseases. The description which follows is a review of some of the discoveries that have been made in connection with the inclusion bodies and virus diseases and also the presence of inclusion bodies in conditions where the presence of a virus is not likely.

Trachoma

Julianelle (96) did conjunctival scrape smears on 602 patients with trachoma and by using either Giemsa or Wright stain was able to demonstrate inclusion bodies in approximately one out of every three patients. This figure closely approximated that found by Julianelle in analyzing 42 reports in this connection. A grand total of 5,777 patients were recorded from various countries of the world, with inclusions occuring in 1,784 or 30.8 per cent.

The observations of Julianelle also revealed that in certain instances, inclusions were present in preparations from one eye only, despite clinical activity in both. In genuine monocular trachoma, inclusions were associated with only the affected eye.

Follow-up smears showed that half the patients with trachoma up to six months duration exhibited inclusions; from then on the incidence fell gradually to zero at about ten years duration. In recurrent trachoma, inclusion bodies appeared in approximately the same frequency as in primary trachoma of recent onset.

To determine the infectivity of trachomatous tissues, Julianelle innoculated 70 monkies with individual tissues from 37 patients, all containing inclusions. Typical infection occured in 70, or 5 per cent of the animals tested. On the other hand, 158 monkeys were innoculated with separate tissues, apparently lacking in inclusions, from 112 patients, and of these animals, 35 or 22 per cent were infected specifically. The pooled tissues were derived from 89 patients, of whom 44 were found to have inclusions. The pooled material was innoculated into 106 monkeys and of these, 41, or 38 per cent, were infected. Thus equal mixtures assumed a mathematically half-way position of infectivity. This may indicate that the inclusion may be a mass of infectious units.

So consistant are the inclusion bodies in

trachoma that Braley (99) establishes a means of differentiation of trachoma and inclusion conjunctivitis on the basis that typical inclusions found in scrapings from the upper lid are diagnostic of trachoma, and when the majority of the inclusions are found in scrapings from the lower lid it is diagnostic of inclusion conjunctivitis.

Braley (99) has shown that several types of intracellular bodies which can be confused with virus inclusions occur in conjunctival epithelial cells. A number of these are normal structural alterations in the epithelial cell:

Granules, called mitochondria, are contained in all epithelial cells. Differentiation from the elementary inclusion bodies of trachoma or inclusion blenorrhea may be difficult by the inexperienced.

Overstaining of normal goblet cells may be interpreted as inclusion bodies.

Melanin pigment granules, when appearing in large amount may be confusing.

Extrusion of nuclear chromatin into the cytoplasm, phagocytosed cellular debris and extracellular bacteria- all may give the illusion of inclusion bodies to the inexperienced.

Thygeson and Stone (93) made a study of 50 cases of inclusion conjunctivitis in infants, children and adults. They demonstrated the virus by miero and Baboon innoculation and found that the reservoir of the virus is a mild genito-urinary disease which is transmitted venereally and in which a low grade urethritis is the lesion in the male and a low grade cervicitis is the lesion in the female. Birth canal, swimming pools and perverted sexual intercourse were means of transfer. Mc Kee (102) substantiates these findings.

Carcinoma

Stein (92) reports a case of primary carcinoma of the adrenal in a fifty-eight year old woman. Histologically the parenchyna of the parenchyma of the adrenal tumor consisted of rounded granular cells which did not resemble the zona glomerulosa or zona fasciculata, and the stroma was very vascular. The malignant cells invaded the connective tissue and in the lumens of many blood capillaries, particularly in the capsule, tumor cells were present. In almost all of the carcinoma cells there were present acidophilically staining, large circular inclusion bodies. These

bodies were conspicuous and many of the larger ones were surrounded by a clear, hyaline-like, unstained halo. Within the section various stages of the development of these bodies could be seen, starting as small discrete masses without a halo to larger ones. Dispersed throughout the connective tissue stroma were a number of polymorphonuclear leukocytes, plasma cells and lymphocytes.

Stewart (106) reports the presence of inclusion bodies in a case of carcinoma of the thyroid gland. These inclusion bodies demonstrated acidophilic staining, halo formation, and margination of chromatin with or without displacement of nucleoli.

A survey of 38 cases of thyroid carcinoma disclosed the presence of intranuclear inclusions in 10.

Nervous System

Smith, Lennette, and Reames record a case of acute encephalitis (100) in which the virus of herpes simplex was isolated by grinding a piece of cortex, from the brain of the diseased infant, in a small amount of nutrient broth.

After light centrifugation, the supernatant fluid was innoculated intracerebrally into Swiss mice. The infective agent was maintained by serial innoculations of Swiss mice following the death of those previously innoculated.

An attempt to grow the infectious agent in dextrose infusion broth both aerobically and anaerobically proved that no bacteria were present.

No alteration of pathogenicity of the organism after passage thru a Berkefeld filter proved the filterable character of the virus.

Neutralization tests showed that the virus was immunologically identical with a known strain of herpes simplex virus.

Histological examination of sections from the pons, medulla and cerebellum stained with hematoxylin and eosin and phloxine-methylene blue demonstrated some cells showing pyknotic nuclei and deeply stained, shrunken cell bodies; others were poorly outlined and showed varying degrees of karyolysis. Many cells, however, showed more specific nuclear changes in the form of intranuclear inclusions which varied somewhat in appearance.

A variety of laboratory animals were innoculated with the Virus. Mice, guinea pigs, and

rabbits. Although succombing to the virus, they did not display inclusions on sections stained identically to those from the human. Rats and chick embryo, however, demonstrated intranuclear inclusions.

Negri bodies (from 98) were discovered by Negri in 1903 in the ganglion cells of rabid animals. Negri believed that these peculiar inclusions were protoqoan parasites. Grinker (98) has isolated virus inclusion bodies and by innoculation into susceptable animals has found them to be non-infective.

By using Mallory's eosin methylene blue stain, Black (98) has demonstrated the striking similarity between the nucleoli of certain neurogli cells of the brain, Negri bodies of rabies, and the intranuclear inclusions of poliomyelitis. Each with a red matrix and containing a characteristic single basophilic granule.

Genital_Tract

Broadhurst, Ewing, Le Moyne and Mac Lean report the incidence of cytoplasmic inclusion bodies in 350 vaginal smears (97). These patients were

divided into three groups: Group A was children under 12 years of age; Group B was smears from 174 adult females, unselected cases, examined routinely in a gynecological clinic; Group C was used to study the incidence of cytoplasmic inclusion bodies in relation to clinical diagnosis. In Group A of 40 patients with no gynecological condition, 7 had inclusion bodies; In Group B 23 per cent of the smears contained inclusion bodies. In Group C, 73 per cent showed inclusions. Of this last group 48 per cent had inclusion bodies whose pathology was non-pelvic. Of the rest, the following conditions showed the ratio of inclusion bodies which follows their heading: Infections of urinary tract 2:0, Vaginal conditions (vaginitis, prolapse, etc.) 30:0, Urethral conditions (urethritis, caruncles, etc.) 15:3, Cervical conditions 3:6, Adenexal conditions 11:4, Dysmenorrhea 20:2.

Gilmour (109) in a series of microscopic sections of over 100 male inclusions in the columnar cells lining the part derived from the Wolffian duct- that is, in the part beginning

with the canal of the epididymis and ending in the common ejaculatory duct. They were not present in males in whom spermatogenesis had not taken place. In those in which spermatogenesis had taken place inclusion bodies were found 100 per cent in the lowermost ends of the vas defrens and the canal of the epididymis.

The inclusions were round and of dense homogenous structure, and stained deeply with eosin. As a rule they were closely bounded by a delicate capsule of chromatin.

Miscellaneous

Semroth (103) reports a fatal case of prodromal measles. At autopsy the epithelium of the respiratory passages showed degenerative changes associated with proliferative ones. The latter consisted in the appearance of amitotic multinucleate epithelial cells comprising cytoplasmic inclusion bodies. They were eosinophilic in character and usually globoid or oval. As a rule they were situated at the basal side of the cluster of nuclei.

Sections of molluscum contagiosum lesions, stained by Mann's method exhibit typical acido-

philic inclusion bodies within the cytoplasm of infected cells. (104).

Broadhurst et al (105) reports the presence of inclusion bodies in the cells of the upper respiratory area of scarlet fever patients: in the surface membranes of the nose, throat and tongue. These smears were stained with nigrosin stain. Blood smears, stained with methylene blue, demonstrated inclusion bodies in the white blood cells of these patients.

Blackman (110) reports histologic studies of twenty-one cases of lead poisoning occuring in children. Intranuclear acidophilic inclusion bodies were found in the tubular epithelium of the kidneys and in liver cells. Also similar inclusions were reproduced in the kidneys of guinea pigs, mice and rats by adding lead to the diet of these animals.

The inclusion bodies were variable in size and shape. Many of them quite lar ge, round, smooth and homogenous in shape. Some were plastic and assumed the shape of the distorted nuclei in which they occurred. Some of the nuclei contained many small granular and drop-like inclusion bodies.

The majority stained with eosin and phloxin and gave negative staining reactions for thymonucleic acid.

Olitsky and Harford (107) injected various autoclaved materials subcutaneously into guinea pigs and subsequently removed the nodules produced for histologic examination. Suspensions of normal brain tissue, normal rabbit tissues, commercial lecithin, alcoholic extract of monkey brain, phosphotide of tubercle bacilli and dibenzanthrene were used. Of these only brain induced characteristic intranuclear inclusions. Aluminum hydroxide injected into other sites than the subcutaneous tissues of guinea pigs produced similar reactions with inclusions.

Composition

Chemical composition of the inclusion body was studied by van Rooyen (104), who made sections from the lesions of Molluscum Contagiosum. Films were gently fixed by heating or with alcohol, were treated with undiluted Lugols iodine solution for one minute, washed with distilled water and examined under the microscope. The inclusions assumed a dark brown colour identical with that of

liver cells after similar treatment. The brown colour so produced could be made to disappear by gently warming the slide and it reappeared on cooling. A film of liver tissue similarly treated behaved in a similar way; this suggests that the molluscum body contains a substance which is glycogen or some similar carbohydrate.

If a film of suitable tissue is fixed by heat and treated with fresh human saliva for 24 hours the iodine- staining substance disappears. A control slide exposed for 24 hours to saliva which has been previously boiled for one hour shows the same appearances as an untreated slide. This strongly suggests that the reacting material is carbohydrate in nature since it can be removed by exposing it to the action of the enzymes, amylase, and ptyalin of saliva.

The ash remaining after autoclaving, although very small in amount, appears to be calcium.

Rice (115) demonstrated by the use of an iodine potassium solution,for staining the fixed smears of epithelial scrapings from trachomatous conjunctivae, a distinct epithelial cell inclusion that seems to contain a very appreciable amount of a carbohydrate

that reacts with iodine in a manner characteristic of some of the polysaccharides. This polysaccharide is thought to be glycogen. The inclusion is acted upon by the enzyme of saliva.

Inclusion bodies from psittacosis and lymphogranuloma venerum did not show positive carbohydrate reactions.

In 1937 Rhodes and van Rooyen (108) infected cultures of tissue culture with vaccinia virus and inclusion bodies developed in fibroblasts, which increased in number and attained a large size.

The tissue culture of course was free of blood cells; thus the bodies cannot have represented the acidophilic granules of degenerate polymorphs. This indicated that the inclusion bodies represent aggregates of actual particles of vaccinia virus, possibly surrounded by a localized acidophilic change in the fibroblastic cytoplasm.

Black (98) has reviewed the extensive morphological studies of Paul and Schweinburg which suggest that the negri body is a vegetable parasite. its characteristic morphological appearance, with a readibly demonstrable internal structure, speaks against its being an ordinary virus inclusion body.

This may be said of many other virus inclusion bodies. Their characteristic appearance with a conspicuous and typical internal granule suggests that they are more than just an amorphous conglomerate red staining mass such as might occur following degenerative changes of the cell.

Rake and Jones (114) describe a regular cycle of development for the lymphogranuloma venerum. inclusion body. The strain of lymphogranuloma was obtained from infected lymph nodes and innoculated into the yolk sac of six day old chicken embryos. Several hundred examinations were made.

Innoculations were made from material which contained elementary bodies. These disappeared and new bodies appeared which at 10-12 hours after innoculation are twice as large as elementary bodies. During the next 6 hours these bodies increased in number (up to 40 or more) and size and came to occupy small vesicles in which they were imbeded in a thin matrix. Between 18-20 hours the bodies increased more in size and formed large plaques which showed vacuoles. These large bodies disintegrated, usually within 30 hours and elemen-

tary bodies were set free, entered new cells and repeated the cycle. Using Nobles' stain the elementary bodies stain red. These red staining bodies disappear and the new phase, the initial body stains green early. As the initial body grows, its green staining matrix is studded with red staining granules- the elementary bodies.

Bedson and Bland have called attention to different developmental forms in psittacosis (116). They made their observations from histologic section of the spleens of infected mice. The elementary bodies stained deep purple with Giemsa and larger bodies were next observed which stained light blue with Giemsa.

Discussion

In reviewing the above literature on inclusion bodies, a striking thing is their highly irregular character.

Most nuclear inclusions are acidophilic, although there are exceptions. Cowdry (95) calls attention to the presence of basophilic nuclear inclusions in the salivary glands of moles.

Chemical composition varies. The inclusions of trachoma consistently contain carbohydrate. In

clusions of guinea pig's submaxillaries are positive with Millons' reagent. Lipids have been reported in inclusion bodies of unknown origin (105). Mineral content is variable (95,104).

The nuclear inclusions would seem to include: Herpes and lymphogranuloma. Cytoplasmic inclusions: rabies, vaccinia, molluscum contagiosum, trachoma and inclusion blenorrhea. Those demonstration both: smallpox and atypical pneumonia?

The actual significance of the inclusion body is baffling. Rhodes and van Rooyen demonstrated inclusion bodies in the fibroblasts of tissues, from tissue culture infected with vaccine, so that the inclusion bodies in this case were not degenerate polymorphs.

The presence of inclusions from tissues not infected with viruses, as the cases of lead poisoning and normal brain tissue reviewed, lends some support to the degenerative theory, i.e. that the inclusions are degenerative products of the cell.

Demonstrations by Rake and Jones (114) in lymphogranuloma and by Bedson and Bland (116) in psittacosis of a developmental cycle of the inclusion body indicates a parasitic nature. They postulate that the parasite may carry the infecting virus.

Perhaps all theories may be combined by assuming a multiple cause of inclusion bodies, and especially in view of their multiple properties.

The difficulty in distinguishing the inclusion bodies from natural and other substances as emphasized by Black and Braley, should make us critical of not only ourselves, but of the reports of others.

D. The Hyaline Membrane.

The occasional occurance of a hyaline membrane in atypical pneumonia (14) warrants a more thorough consideration of this finding.

A peculiar eosin staining membrane, the so-called hyaline membrane, lining the walls of the bronchioles and alveoli was noted in the lungs of patients who died of influenza in the epidemic of 1918.(85) This formation was considered pathognomonic of the disease. This idea remained untill 1932 when Farber and Wilson (91) found identical membranes in the lungs of persons with various types of pneumonia and in the lungs of new-born infants after aspiration of amniotic sac contents. They concluded that the hyaline membrane was not pathognomonic of influenzal pneumonia.

Winternitz (117) particularly noted the hyaline membrane in pneumonia where the lungs at autopsy were airless and distended with a bloody, serous fluid. The pleural surfaces were mottled with brilliant colors. Occasional large areas of hemorrhage suggested infarcts and emphysema was often a striking feature, extending into the mediastinal tissue.

In literature dealing with the hyaline membrane there is a marked lack of agreement in regard to the significance of the picture, the composition of the membrane and the mechanism of its formation. It has been variously described as a fibrin or a fibrinoid deposit, the "lesion of characterization" of influenza, and the "virus lesion" distinct from the changes due to secondary pathogenic invaders.

The various explanations regarding the mechanism of the formation of the hyaline membrane can be divided into three main groups: 1. That in which the membrane is regarded as the result of hyalinization of necrotic alveolar walls; 2. That in which it is considered to be the result of injury to the capillary bed and 3. That in which it is held to be the result of air forced into the exudate.

Winternitz and his associates in a series of communications (117, 118), attributed the membrane to hyalinization of the necrotic material resulting from the action of some powerful agent on the alveolar walls. The described the membrane as a swollen homogenously staining material, quite without architecture, replacing the alveolar epithelium. They stressed the generalized congestion, the hemorrhage into the pulmonary parenchyma and the albumineus,

rich serous exudate that occupies the foreground in the picture of the acute disease. These features were observed in patients who had suffered from intense cyanosis, dyspnea and pulmonary hemorrhage. The acute necrosis, which involves first the epithelium of the trachea, bronchi and bronchioles, and which may extend beyond the epithelium into the walls of these structures or may even destroy en Masse the walls of the alveoli, causes a lesion which they believed occured characteristically in influenza and could not be brought about by other types of pulmonary infection. They did believe that the action of various war gases on the lungs produced effects similar to those seen in influenza. Similar changes after inhalation of war gases were noted by German workers by the name of Kucznski and Wolff, according to Winternitz.

Brannon and Goodpasture (119) in 1924, suggested the possibility that the hyaline membrane was the result of damage caused by a circulating toxin which occurred in cases of severe influenza and which caused mild injury of variable degree to the capillary bed of the lungs. They believed that this injury to the pulmonary capillaries permitted an exudation into the alveolar spaces of fibrinogen-containing serum, and

that from this exudate fibrin would quickly form, adhere to the alveolar walls, and become fused, thereby forming a "membrane".

Wolbach (4), and later Opie, Blake, Small and Rivers (5), offered the suggestion that the hyaline membrane was related to the action of air on some of the body fluids or exudates. Wolbach described the membrane as being closely applied to the walls of the alveoli and alveolar ducts, and as sometimes apparently replacing them, but in other areas as being actually separated from these walls by desquamating epithelial cells or by red cells and leukocytes. He believed that the formation of the membrane was not dependent on the presence of necrotic alveolar walls. Wolbach concluded that the arrangement and outline of this hyaline material were determined by its contact with air, which must be under some tension.

As has been stated earlier the hyaline membrane at one time was regarded as pathognomonic of influenza, and it has been described as the distinctive lesion caused by the virus of influenza. In 1924 Brannon and Goodpasture (119) reported two instances of the occurance of hyaline membranes in the absence of any association with influenza.Johnson(122)

in 1923, quoted a personal communication from Professor Hayashi of Japan to the efect that the hyaline membranes were observed in pneumonic plague.

In 1931 Farber and Sweet called particular attention to the occurance of similar membranes in the lungs of newborn infants with a birth history suggestion intra-uterine asphyxia with aspiration of amniotic sac contents. The term "vernix membrane" was applied to these structures. These membranes were indistinguishable in appearance, position and ordinary staining reactions from those they had observed in influenza.

Farber and Wilson (91) in 1932 reported vernix membranes in over fifty new-born infants and typical hyaline membranes in the lungs of twenty infants and young children, varying in age from three months to four years, whose deaths were caused by pneumonia due to infection with streptococcus hemolyticus. In addition, hyaline membranes were found in the lungs of a young child who died of acute tuberculous pneumonia, a young adult who died of streptococcic bronchopneumonia and a man of fourty two whose case ran the typical course of lobar pneumonia. In the last case, numerous membranes were found in a less involved por-

tion of the lung.

The Composition of the Hyaline Membrane

Farber and Wilson (91) describe the typical membrane as consisting of a homogenous amorphous material, in which cellular debris and bacteria can easily be found. Both in cases of influenza and lobar pneumonia studied the appearance of the material in the membrane is that of fused, resolved exudate consisting of necrotic mononuclear cells, leukocytes and erythrocytes, altered fibrin and serum and debris, from the alveolar walls, when necrosis of thses walls is present. The material in the membrane is comparable to the exudate in lobar pneumonia during the stage of resolution when autolysis of the exudate is taking place and cellular detail can no longer be identified. Under these circumstances, former staining reactions are lost, and the necrotic materials stain deeply with eosin (91). A similar change characterizes the appearance of necrotic debris in an area of tuberculous caseation. When viable cells are present in the membrane, organization is probably taking place, for as Wolbach (120 b.) showed, during repair there is invasion of the membrane by leukocytes and phagocytes. Eventually organization takes place in the membrane.

The membrane found in the lungs of new-born in-

fants (vernix membrane) consists of amorphous material, embeded in which can be found cornified epithelial cells. It was shown by Farber and Sweet (124) that this type of membrane, identical in appearance and staining reactions with the hyaline membrane, consists of aspirated amniotic sac contents. The bulk of the membrane is composed of vernix caseosa. There is no evidence of necrosis in association with the vernix membrane.

Farber and Wilson (91) conclude from their staining reactions that the hyaline membrane in influenzal pneumonia, the membranes associated with the various types of pneumonia, and the vernix membranes have the same staining reactions. They vary from pink to red with hematoxylin and eosin and from salmon pink to red with phosphotungstic acid hematoxylin stain. Scharlach R stains reveal the presence of fat in the membranes which is variable in amount. The vernix membranes can be differentiated from all other hyaline membranes by the cornified epithelial cells from the amniotic sac contents embeded in the vernix membrane. These can usually be identified in the ordinary stains. The Gram stain followed by acid alcohol decolorization may prove useful in doubtful cases (91). Although altered or

digested fibrin is without a doubt one of the components of the membrane, special stains such as phosphotungstic acid hematoxylin fail to reveal the altered fibrin.

There is no record in the literature of anyone determining the exact chemical nature of the hyaline membrane in the lung but most pathology textbooks subdivide hyalin, as does Karsner (126), into mucin, colloid, glycogen, amyloid and several other clear structureless materials.

Mucin differs somewhat in microscopic appearance, depending on precipitation by the fixing agent. At times mucin appears under the microscope as a finely granular material and again as a structureless hyaline material. It can be differentiated from other forms of hyaline by virture of the fact that it takes the basic dyes (126). Mucin has been described (126) as a "compound protein consisting of a protein radical and a conjugated sulfuric acid which contains a nitrogenous sugar".

Colloid actually should not be considered a hyalin material because it differs from the other types in containing iodin. Its counterpart called pseudomucin does not contain iodine and may be considered true hyalin. Colloid and pseudomucin histologically take

the acid stain and show an entirely structureless hyaline character. Chemically they are considered to be a condensation of protein material.

Glycogen is a carbohydrate and so is dissolved by ptyalin, even after fixation. In order to demonstrate glycogen in tissue sections, it is necessary to use fixatives, such as alcohol, or watery fixatives saturated with dextrose, in which glycogen is not readily soluble. Embedded in paraffin or celloidin, it is stained brown with iodine. Its intracellular position distinguishes it from amyloid.

Amyloid is a protein complex (126) which takes the acid stain. Grossly, amyloid is homogenous and glassy in appearance and transmits the color of the organ in which it was found.

Mechanism of Formation of the Membrane

Any explanation of the formation of the membrane must take into consideration not only the membrane seen in influenzal pneumonia, but also that found in various other types of pneumonia and the membranes occuring after aspiration of amniotic sac contents.

The outstanding clinical and pathological features of cases of influenzal and streptococcic pneumonia are marked dyspnea (with emphysema) in addition

to the presence of a large amount of semifluid material in the air spaces. Similar features characterize cases of aspiration of amniotic sac contents. Small and large masses of eosin staining amorphous material are found free in the alveolar spaces both in the persons who died of pneumonia and in the new-born infants. Farber and Wilson (91) regard these masses as earlystages in the formation of the membrane before the material has been pressed against the alveolar walls and that a sequence of events leading to the formation of a membrane can be followed. This sequence can be traced from the coalesence of small masses of amorphous eosin staining material, free in the alveolar ducts and later in the alveolar spaces, with dispersion toward the periphery to an ultimate close application of the material against the alveolar walls in characteristic membrane formation. This sequence is similar to that noted after aspiration of amniotic sac contents with formation of the vernix membrane (124). The dispersion of the coalesced amorphous materials to the periphery, the characteristic picture of such substances surrounding bubbles of entrapped air, and the occurrance of typical membranes in the mediastinum in the presence of

mediastinal emphysema and exudate all demonstrate a relationship of air to the formation of the membrane- a relationship emphasized by Wolbach (120) and later by Opie, Blake, Small and Rivers(121).

In the light of these observations it seems that the necessary factors for the production of the characteristic hyaline membrane are: 1. The presence of material capable of taking the characteristic eosin stain. 2. Air in the alveolar spaces, probably under greater than normal tension. 3. Partial obstruction to the passage of air by semifluid material in the air passages and 4. Dyspnea, which may be interpreted as violent respiratory efforts in an attempt to force air by this obstruction.

Farber and Wilson (125) believing that the position of the eosin staining substance lying characteristically as a membrane along the alveolar walls must be simply the result of the mechanical dispersion of this foreign material toward the periphery by air violently inspired, made the following experiments:

. Asphyxia With and Without Dysphea: a series of rabbits was placed in small, air-tight glass containers and allowed to remain untill death from

asphyxia occurred. The jars were of such a size that two and one-half hours elapsed before the oxygen was sufficiently used up to produce death. Marked dyspnea resulting from the accumulation of carbon dioxide was present for much of the time.

A second series of animals was allowed to die in a similar manner, except for the constant removal of carbon dioxide by soda lime. In thes animals there occurred only a small amount of terminal dyspnea. At autopsy, the lungs of the first series of rabbits which died with evidence of marked dyspnea, were dark salmon pink externally. On section, the cut surfaces showed marked congestion, and large amounts of blood flowed freely from the larger vessels. Serum could be readily expressed from the parenchyma on pressure. Microscopically, the blood vessels showed marked congestion, and alveoli were definitely distended and contained varying amounts of serum, often pressed against alveolar walls in membrane-like formation. The lungs of the second group of rabbits, which died without much dyspnea, showed no distension of the alveolar spaces and only small amounts of serum in these spaces. No membrance formation had occurred.

Aspiration of Ink with Resulting Dyspnea: A number of young rats were immersed in a solution of 10 percent india ink for from one to two minutes. They were then removed and permitted to breathe with marked dyspnea for a few minutes befor they were killed. At autopsy, the ink could be seen distributed throughout all lobes of both lungs. Microscopically, the alveoli contained large amounts of the ink solution, sometimes gathered in small patches without definite shape, free in the air spaces, but more often forming eomplete rings around the air spaces lining the walls and often sealing off groups of alveoli. These membrane-like formations were usually not very wide and were characteristically formed around entrapped bubbles of air. The india ink membranes were an exact duplication of the hyaline membrane of influenza in shape, position and general outline, and differed only in staining reaction.

Aspiration of Foreign Material During Artificial Respiration: In order to produce even more excessive respiratory movements in the presence of obstructing fluid matter in the air passages than an animal would produce by itself, a small improvised Drinker respirator was utilized. The animals were placed in the respirator after anesthesia and subjected to violent

artificial respiration (vacum up to 30 cm. of water press.). While artificial respiration was maintained, horse serum, india ink and various types of purulent material were injected into the trachea of these animals. Respirations were maintained in the Drinker apparatus for varying intervals of time, and then the animals were killed. Cats, rabbits and puppies were used for these experiments. In every case, the material, could be found in the alveolar spaces. The foreign matter was usually widely dispersed throughout all the alveoli, sometimes collecting in small patches, but more often forming typical membrane-like sturctures against the alveolar walls. The most striking pictures were obtained after the injection of india ink, because of the outstanding color of the ink. However, in all cases, the same type of formation as regards shape and position could be found.

Insufflation of Hydrochloric Acid, with Vascular Damage and Dyspnea: Several rabbits were subjected to insufflation of hydrochloric acid, in imitation of the experiments of Winternitz and his co-workers; 7 cc. of a one per cent solution of concentrated hydrochloric acid in physiologic solution of sodium chloride was injected intratracheally. Immediately

the respirations became of a noisy, obstructive type, and diffuse fine rales could be heard over the lungs with the stethoscope. Marked dyspnea was brought about. Frothy, serosanguineous fluid flowed from the mouth during the last few minutes of life. At autopsy, the mucosal surface of the trachea was reddened and covered by foamy, serosanguinous fluid exudate. The lungs showed patches of consolidation, and numerous hemorrhagic areas of varying size and shape and could be seen beneath the pleura. On section, the cut surfaces were edematous and hemorrhagic. Microscopically, the main features were edema, and hemorrhage into the alveoli. The alveolar spaces were filled with large amounts of serum and smaller amounts of red cells. The blood vessels showed marked congestion and early thrombi. In scattered areas, serum was packed against the alveolar walls in membrane formation. In two areas, membranes reminiscent of the hyaline membrane of influenza were seen lining alveolar walls, which showed early necrosis. The membranes consisted of loose fibrin, serum and a small amount of necrotic debris. Distension of the alveolar spaces was a marked feature. The vascular lesions and the marked amounts of serum and blood in

in the alveoli overshadowed the comparatively small amount of necrosis of alveolar walls in animals treated by insufflation of hydrochloric acid.

Experiments Showing Exudate as an Essential Factor: Several experiments were devised to show the necessity for the presence of an exudate as well as of air under some pressure in order to produce a membrane. A cat was overventilated to such a degree that mediastinal emphysema resulted. However, no exudate was present in the mediastinum, and on section, although mediastinal emphysema was demonstrated, there was no membrane formation. In contrast to this situation, air and purulent material obtained from other sources were both forced into the areolar tissues beneath the skin of a rabbit. On section, the air bubbles were surrounded by the fibropurulent exudate that had been injected. Typical membrane-like formations occurred in this case, of characteristic hyaline appearance.

Experiment Further Demonstrating Mechanical Nature of Formation of the Membrane: To ascertain whether the living animal was necessary for the formation of the membrane, two rabbits were killed and india ink was injected intratracheally when the animals were in the

Drinker respirator. Artificial respiration under increased pressure was then instituted. Microscopically, a picture just as striking as that seen after insufflation of india ink in the living animal was found. Ink membranes in characteristic position and outline could be found in large numbers throughout the lungs.

Source of Fat in Membranes

The following experiments were carried out by Farber and Wilson (125) to determine the origin of the fat found in the membranes of a small percentage of cases:

Experiment to Determine Whether Fat Could be Drawn From the Blood Stream Under Varying Conditions: Puppies, rabbits and rats were placed in the Drinker respirator and subjected to prolonged overventilation with varying degrees of pressure. In all cases there occurred marked distension of alveolar ducts and spaces with occasional rupture of the alveolar walls. Serum and small collections of red cells, usually pressed against the walls of the air spaces, could be found. Fat stains (Scharlach R after formaldehyde fixation) failed to reveal the presence of fat in the air spaces in any of the animals treated in this manner.

Experiment to Ascertain Whether Fat Could be Brought from the Blood Stream into the Alveolar Spaces when the Blood was Rich in Fat: Four cats were fed heavy cream at varying intervals with the hope of temporarily increasing the fat content of the blood stream. Procedures similar to these just described were carried out following which the animals were killed. The lungs of these cats showed emphysema, overdistension of the air spaces and small amounts of serum in the alveoli, usually pressed against alveolar walls. Globules of fat were present in the blood vessels of the lungs or in the peribronchiolar lymphatics, greatly in excess of the amounts usually seen. However, no fat could be demonstrated in the alveolar spaces.

Other Experiments in which the Source of Fat was Studied: In the experiments in which horse serum was injected into the trachea and the animal then subjected to violent respiratory movement, no fat could be demonstrated in the resulting membranes. Staining for fat was done on the lungs of all the animals subjected to any of the experimental procedures mentioned in the foregoing paragraphs, and in no case could fat be demonstrated in the alveolar spaces. With death caused by overventilation, immersion followed by dyspnea, or asphyxia accompa-

nied by hyperpnea and dyspnea or without dyspnea, stains for fat always gave negative results. After the insufflation of various substances fat-free in themselves, similar negative results prevailed.

Evidence that Fat is from Exudate: The only other explanation of the presence of fat in the membranes in certain of the cases of streptococci and influenzal pneumonia must depend on the production of fat from the purulent exudate in the lungs. There is abundant evidence for this supposition. In 1,000 parts of dried pus cells, there are 75 parts of fat and like amounts of lecithin and cholesterol. In addition, pus-containing serum shows approximately 1.2 per cent fat, cholesterol and lecithin, with fat representing 0.5 per cent (Wells- from 125) Small amounts of fat are present in any pneumonic exudate (85).

From histologic appearances and staining reactions it may be concluded that the eosin staining reaction of the hyaline membrane is due to the nature of the exudate and the debris included in the membrane and to the degree of autolysis which the exudate has undergone.

Fibrinopurulent exudate from a case of emphysema was mixed with a physiologic solution of sodium

chloride by Farber and Wilson (125) and injected into the tracheas of one live and one dead rabbit, while the animals were subjected to forceful artificial respiration in the Drinker apparatus. Microscopically, the exudate was found in the alveolar spaces of both animals, often in membrane formation against alveolar walls. The membranes consisted of leukocytes and fibrin, and only in occasional small areas were they typically eosin stained. Similar exudate was then allowed to stand in an incubator at 40 degrees centigrade for two days, following which the material was injected as before into the tracheas of both dead and live rabbits, which were subjected to artificial respiration. Microscopically, the material was found scattered throughout the air spaces in all lobes of both lungs. Material was gathered, in part, in small patchy areas in the alveolar spaces and ducts. More often, however, the injected material was found pressed against alveolar walls in amorphous masses in which no cellular detail could be recognized. This material stained definitely pink to red in hematoxylin and eosin stains, and by phosphotungstic acid hematosylin stain was negative for fibrin. The picture could not be distinguished from the hyaline

membrane found so characteristically in influenzal pneumonia.

A summary of material correlated so far emphasizes the presence of membrane-like structures lining alveolar walls and identical in position and shape but not in staining reaction with so-called hyaline membrane of influenza were found, on histologic examination, in the lungs of patients dying from a variety of causes. The presence of fluid matter in the air passages, causing partial obstruction to the passage of air, and marked dyspnea characterizing the deaths of these patients.

The fat found in the membranes in certain cases of influenzal and streptococcic pneumonia is best explained as fat liberated from the exudate as a result of autolysis. It was found impossible to draw fat into the alveolar spaces from the blood stream by reproducing in a number of ways the important mechanical factors that are present in every case in which the membrane is found.

When animals were forced to breathe an atmosphere of such low oxygen and high carbon dioxide that marked dyspnea leading to death occurred, the alveolar spaces were filled with varying amounts of serum frequently pressed by the inspired air into membrane

formation against the alveolar walls. When carbon dioxide was removed so that little dyspnea occurred before death from anoxemia, serum was found in the alveoli but no membrane formation.

When a foreign substance, such as horse serum, india ink or fibrinopurulent exudate, was instilled into the trachea and vigorous artificial respiration (in a Drinker respirator) instituted, characteristic membranes formed of the material could be found lining the alveolar walls.

That the living animal is not necessary for the production of the membrane, a nd that only the correct mechanical conditions are of importance, was shown by the production of membranes in the lungs of dead animals sugjected to artificial respiration during the intratracheal injection of various foreign materials.

The absence of membranes in a mechanically produced mediastinal emphysema where no exudate was present, and the production of such membranes in artificial emphysema caused by the injection of both air and exudate into the subcutaneous tissues of an animal, stress the necessity of having both factors present for the formation of a membrane.

The eosin staining character of the typical membrane, as seen in influenzal and streptococcic pneu-

monia, was found to depend on the degree of autolysis of the exudate incorporated therein. It was possible by injecting suitable exudate into the tracheas of dead animals subjected to artificial respiration to produce typical eosin staining membranes lining the walls of bronchioles and alveolar spaces.

Since the work of Farber and Wilson, the subject of hyalin has been of little concern to American investigators.

Ophthalmologists have been interested in the appearance of Hyaline Scleral Plaques. The most common location of the Plaques is opposite the medial rectus(127). There seems to be a connection between polyarthritic diseases (rheumatic, rheumatoid and gouty) and Scleral Hyaline Plaques. Out of eleven patients reported by Boshoff (127) only one was free of objective signs of arthritic involvement, but he had vasosclerotic changes. Graves (130) reports similar findings, and so does Culler (131).

These Plaques appear in an area of the sclera where the blood supply is poor, so that it is probable that hyalinization is directly due to a local

deficiency of blood supply, resulting in a degeneration of scleral fibers of the hyaline type.

At one stage in the development of arteriosclerosis the intima is hyaline in appearance. It has been frequently suggested that the thickened, hyalinized arterioles in human arteriosclerosis may be the result of hypertension causing marked hypertrophy of the muscular coat of the vessel or "hyperplastic sclerosis" of the intima, with subsequent death of the cells from "overstrain", followed by hyalinization of the dead cells. The fact that hyaline arteriosclerosis is found in persons without hypertension suggest that perhaps other factors, such as toxin, may play a part(126).

It is not the purpose of this paper to discuss arteriosclerosis but to complete the discussion on hyaline, some mention of the process seemed pertinent. Also of interest, are two experiments that have been carried out quite recently:

In 1937 Rich and Duff (129) made up sterile solutions of pancreatic duct juice, commercial trypsin, crystalline trypsin, crystalline chymotrypsin, and papain and injected at different

sites into the subcutaneous tissue of normal dogs. The sites for injection were marked with ink and twenty four hours later they were excised, fixed in Zenker-formol and sectioned for histological study. Arteriolar lesions were produced which in all respects had the histological appearance characteristic of human hyaline arteriolosclerosis. The cellular structure of the media of these arterioles disappeared completely, leaving only a homogeneous hyaline substance which was sometimes faintly basophilic, as is not infrequently the case in human hyaline arteriosclerosis, and sometimes purely eosinophilic. In some vessels only a segment, in others the entire circumference was affected. Fat stains revealed no lipoid in the affected vessels.

The other experimental finding was by Daft, Ashburn, Spicer and Sebrell in 1942. Extensive hyaline sclerosis and calcification of blood vessels in seven young rats observed in the course of some preliminary experiments with purified B complex deficient diets containing 1 per cetn sulfaguanidine, supplemented with thiamin, riboflavin, pyridoxine, pantothenic acid, nicotinic acid and choline, and given continuously for 62-192 days. This pathologic change was found in the small arteries of the heart,

lungs, kidney, pancreas, and the submucosa of the intestinal tract.

The location and degree of involvement was variable. The vessel wall was often completely replaced by a homogenous or glossy material which is metachromic or lightly basophilic with eosin and polychrome methylene blue. This glossy material as seen in routine paraffin sections is markedly shattered into variably sized and shaped plates. It forms an orange-brown lake with alizarin red S.

It is impossible to state whether this pathologic condition has its basis in a dietary deficiency induced by sulfaguanidine, orwhether the sulfaguanidine or a compound derived from it has contributed directly to the sclerotic changes.

Summary

It has been shown that the hyaline membrane in the lung can be produced by both infections and mechanical means, although dyspnea has been demonstrated to be a probable essential factor.

The occurrence of hyaline formations in areas of poor blood supply and toxic sites have been mentioned.

III. CONCLUSIONS

Some of the clinical features that have been responsible for calling the type of pneumonia under consideration "atypical" may be partially explained by the pathological findings.

The absence of pain in the chest in many cases may be explained by the fact that pleural involvement is slight and often absent. The cough is due to an associated tracheitis and bronchitis (60) and the fact that it is usually dry is explained by an absence of the great amount of serum found in the bronchial lumina of patients with bacterial pneumonia.

The dyspnea and cyanosis are due to lack of proper aeration which in turn is hampered by the edema, infiltration and overgrowth of the alveoli and their septa.

The atypical type of pneumonia is undoubtedly to be differentiated from influenza pneumonia on the basis of an absence of secondary bacterial invasion in the atypical type.

The main difference from lobular and bronchopneumonia is that there is thickening of the bronchial walls (38), not as much fibrin, and a mononuclear infiltration of the bronchial and alveolar

walls (36) in the bronchopneumonia.

Animal experimentation has shown that there are probably different grades of infection, depending on virulence and the amount of the infecting agent. This is probably one of the reasons for the discrepancies found in various reports.

It is impossible to explain at this time the occurrance of the hyaline membrane and inclusion bodies, although anoxemia seems to be a very important factor in the formation of hyaline.

After reviewing the literature on inclusion bodies and the hyaline membrane it is interesting to note the similarities between the two: Both predominately have acidophilic staining reactions, certain forms of hyalin are known to be made up of carbohydrates or proteins (126) and the same substances have been demonstrated in inclusion bodies (95); also both the bodies and the membrane have early in their history been associated with virus infection and later been produced by mechanical means.

Karsner distinguishes hyalin containing glycogen as occurring intracellularly. It is in the realm of possibilty that the two findings, inclusion bodies and the hyaline membrane, may prove to be one and the same pathological process.

The fact that some of the pathological reports do not mention either negative or positive findings for inclusion bodies, hyaline formation or bacteria would indicate that no effort was made to find them. In this regard it is to be stressed that sections of all pathological material, from atypical pneumonias, should be stained with eosin-hematoxylin stain in order to demonstrate hyalin or inclusion bodies when present. When the hyalin or inclusions are seen they should be further stained to determine their composition (Carbohydrate, fat or protein). It is only from such statistics that more accurate conclusions may be drawn.

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