

## STUDIES UPON EXPERIMENTAL PNEUMONIA IN RABBITS.

### VIII. INTRA VITAM STAINING IN EXPERIMENTAL PNEUMONIA, AND THE CIRCULATION IN THE PNEUMONIC LUNG.\*

BY B. S. KLINE, M.D., AND M. C. WINTERNITZ, M.D.

*(From the Department of Pathology of Johns Hopkins University, Baltimore.)*

It has been shown (1) that when pneumonia is produced in a vitally stained animal, the consolidated area is intensely stained, while the remainder of the lung remains very faintly colored. The colorization of the pneumonic area is dependent upon the localization of the dye in the fibrin of the exudate. The nuclei of some of the leucocytes are also stained.

On the basis of these preliminary experiments it was decided to test more carefully the affinity of the dye for the various elements of the exudate at intervals in the progress of pneumonia. In the course of this study phenomena presented themselves which led to a more detailed examination of the circulation in the pneumonic lung.

#### INTRA VITAM STAINING IN EXPERIMENTAL PNEUMONIA.

The stain used in the experiments was trypan blue. The method of injection was the same as that described in a previous paper (2).

As is well known from the studies of Bouffard (3), Goldmann (4), and others, the tissues of the lung have very little affinity for the dye, and in normal, vitally stained animals only a few of the cells of the interstitial tissue contain blue granules in their protoplasm. Consequently, the lung appears pale pink in contrast to the liver, for example, which is intensely stained.

Schulemann (5) and others have pointed out that the cells of the circulating blood do not absorb the dye, and the only cells that may appear in the blood containing it are huge macrophages similar to those found in the serous cavities of the body. These cells are not found in the pneumonic exudate which is composed of blood cells, chiefly polymorphonuclear leucocytes.

\* Received for publication, January 23, 1915.

It has been shown in the unpublished experiments of Evans and Winternitz that the nucleus of a living cell never stains vitally, but that as soon as a cell is injured its nucleus absorbs the stain. In the hands of other observers, Evans and MacCurdy (6), this fact has been successfully used as a guide to the recognition of cell injury when other methods have failed. It was therefore hoped that the fate of the leucocyte in the pneumonic exudate might be determined by this method.

In this experiment forty-one rabbits were used. Some of the animals received one, others two, and still others three intravenous injections of twenty cubic centimeters of a 1 per cent. trypan blue solution. In some cases the dye was given before the intratracheal inoculation of pneumococci, while in others the first dose of trypan blue was given as late as sixty-five hours after the injection of the organisms. The animals were killed at intervals varying from three hours to six and one-half days after the intratracheal injection of pneumococci.

The results of these experiments may be summarized as follows: As early as seven hours after the intratracheal inoculation of pneumococci an occasional polymorphonuclear leucocyte with its nucleus stained intensely blue occurs in the exudate. These apparently dead cells increase in number as the process advances but never form the majority of the cells of the exudate. At first these stained cells have distinctly polymorphous nuclei, but slowly the characteristic shape of the nucleus is lost and it is converted into a small, homogeneous, blue staining mass. Furthermore, as early as three hours after the process has begun, polynuclear cells containing blue granules in their cytoplasm are seen in the alveoli, bronchioles, and blood vessels.

These granules correspond in size, shape, and number to the amphophilic granules of the rabbit leucocytes, and occur in a large number of polynuclear cells at all stages of the disease. As the process advances, in addition to these granules, the cytoplasm occasionally contains within it much larger, irregular, blue masses.

Since vitally stained granules have never been described in polymorphonuclear leucocytes, these findings incited a more careful study of the polynuclear cells in the general circulation of otherwise

untreated, vitally stained animals, and in vitally stained animals in which pneumonia had been produced.

A large number of smears from the ear vein of both these types of animals was studied. The polynuclear cells in these smears were almost constantly unstained; very exceptionally one with blue granules occurred. The significance of this selective staining of the granules of the leucocytes in the pneumonic lung has not been determined.

From the experiments of Winternitz and Evans, it is known that the granules of the polymorphonuclear leucocytes stain with trypan blue when the cell is injured. This injury may be accomplished in many ways; *i. e.*, by mixing the blood with benzol, by pressing the cover-slip forcibly on a fresh blood smear, etc. It is possible, therefore, that the staining of the granules of the leucocytes in the pneumonic lung may result from some injury to the cell membrane. This does not seem probable, since cells are found with only part of their granules vitally stained. The only elements in the pneumonic exudate that stain vitally are these granules and the fibrin. The granules, although at first similar in size and shape to the normal granules of the leucocyte, may become larger and more irregular in shape. It is possible that the staining of these granules may be associated with some functional change within them perhaps related to fibrin formation.

#### THE CIRCULATION IN THE PNEUMONIC LUNG.

When pneumonia is produced in animals with vital stain in their circulation, the involved lung has a uniform blue color; if, however, the dye is injected sometime after the pneumonia is produced (twenty to sixty-five hours), pale gray consolidated areas occur in the otherwise densely blue stained, involved lobes. Such areas occur most frequently at the periphery of the lobe, but there is no uniformity in their distribution. Similar areas were observed in the pneumonic lungs of animals not vitally stained. Here they appear grayish white and are much less granular on section than the remainder of the consolidated lobe. The sharp demarcation of these wedge-shaped, infarct-like areas was so striking in the vitally stained animals that it led to the following study of the circulation of the lung in pneumonia.

The consensus of opinion concerning the progressive pallor of the pneumonic lung after the stage of engorgement is expressed in the more recent text-books as follows: "The gray color is caused partly by the large number of leucocytes in the exudate and partly by the poor supply of blood in the capillaries which are compressed by the ever increasing exudate" (7). "As the exudation increases in amount and the fibrin meshwork thickens, the air-sacs are greatly distended, the alveolar walls stretched, and the capillaries compressed so that they no longer appear engorged. As a result, the redness due to congestion and to some extent to hemorrhage fades and the lung passes into the stage known as grey hepatization" (8).

The purpose of this investigation was to determine, first, whether there is any impairment of the circulation in the pneumonic lung, and, secondly, if so, how it is brought about.

On Dec. 15, 1913, there came to autopsy a patient with lobar pneumonia involving the right middle and upper lobes. Three and a half hours after the patient's death the lungs were injected through the pulmonary artery, under a pressure of 120 mm. of mercury, with a Berlin blue gelatin solution (equal parts of 5 per cent. Berlin blue and 10 per cent. gelatin solution). After the injection the pulmonary artery was tied and the lungs were placed in 10 per cent. formalin over night. On section the following morning a striking picture presented itself. The consolidated area (right upper and middle lobes) was pale gray, whereas the non-consolidated area (lower lobe) was intensely blue. An extremely small amount of the blue solution had found its way into the vessels of the consolidated area. Four subsequent cases, treated in the same manner, yielded the same result. With the same method, a series of rabbit lungs, showing experimental lobar pneumonia, was injected immediately after the death of the animals and on section these likewise presented the same striking contrast.

Microscopic examination of the human and rabbit lungs entirely corroborated the macroscopic findings. Very little of the blue solution was found in the consolidated areas, whereas the blood vessels of the uninvolved portions were engorged with the blue injection mass.

These experiments prove that after death, under exactly the same conditions, the vascular bed in the consolidated area can be but imperfectly injected with the dye as compared with the uninvolved portions of the same lung. It was next necessary to determine whether this circulatory impairment could be explained by the increased intra-alveolar pressure due to the exudate.

Accordingly a normal rabbit was killed with ether and the lungs were removed. A cannula was inserted into the left bronchus and the left lung was distended with air so that its volume was considerably greater than if it had been the seat of lobar pneumonia. While the left lung was still so distended, an injection mass was forced through the pulmonary artery under a pressure of 120 mm. of mercury. There was apparently no difference macroscopically be-

tween the lobes in which the intra-alveolar pressure was very great and those in which it was slight, either in the rapidity of injection or the amount of dye present.

Microscopic sections also failed to show any difference in the quantity of blue on the two sides.

This experiment was followed by another in which the intra-alveolar pressure was increased by the introduction of a 10 per cent. gelatin solution into the left bronchus under a pressure of 120 mm. of mercury. The solution and the lungs were kept at body temperature during the injection. After the gelatin had been forced into the alveoli the lungs were placed in cold water to allow the gelatin mass to solidify, and, when this had taken place, the lungs were injected through the pulmonary artery with a Berlin blue gelatin mass in the manner above described.

On section gross and microscopic examination failed to reveal any difference between the two sides.

A similar experiment was performed, with freshly drawn rabbit blood to distend the alveoli, and the results of the subsequent vascular injection were identical with those obtained when air and gelatin were used to raise the intra-alveolar pressure.

These experiments prove that the impaired circulation in the pneumonic lung can not be due to the pressure of the exudate within the alveoli. An attempt was then made to determine what element or elements of the inflammatory exudate were responsible for this impairment of the circulation.

A number of rabbits was given benzol until their leucocytes were reduced to below 1,000 per cm., and then in each one 5 c.c. of a culture of pneumococci were injected as deeply into a bronchus as the catheter could be inserted. At the end of forty-eight hours the animals were killed with ether. They all showed lobar type of consolidation. With the same method described above the lungs were injected with Berlin blue gelatin.

On section it was found that the consolidated areas were for the most part grayish white in appearance, whereas the uninvolved lobes were colored deep blue. Microscopically the exudate in the consolidated areas was found to consist almost entirely of fibrin. In addition there was a small amount of serum, an occasional alveolar cell, but very few leucocytes. The vessels in these areas contained very little of the blue injection mass and corresponded in this respect to the vessels of the injected pneumonic lung in normal rabbits.

These experiments indicate that the fibrin is the important element in the interference of the circulation in the pneumonic lung, for pneumonia in aplastic animals is characterized by an exudate of fibrin with an almost total absence of cellular elements (9). An attempt was therefore made to produce pneumonia in animals in

which the fibrin formation had been inhibited. This was accomplished through the use of phosphorus and chloroform.<sup>1</sup>

A rabbit was injected subcutaneously with 0.8 c.c. and 0.4 c.c. of an old solution of phosphorus in olive oil, together with 0.9 c.c. of chloroform in 30 per cent. alcohol intragastrically, on two successive days. Twenty-four hours later the clotting time of the blood was found to be greatly delayed, and the resulting clot very soft and jelly-like. Then 5 c.c. of a twenty-four hour culture of pneumococci in pig serum broth were injected as deeply into a bronchus as the catheter could be inserted. The animal died in seventeen and one half hours, and at autopsy showed consolidation of practically the entire left lower lobe. Berlin blue gelatin was then injected through the pulmonary artery.

On section the consolidated lobe was found to be almost as uniformly blue as the uninvolved lobes. Microscopic section showed that the exudate contained an abundance of cells but very little fibrin. Furthermore, the vessels of the consolidated area contained a much greater amount of dye than had been observed in any of the pneumonic lungs described above.

These experiments demonstrate that the fibrin is responsible for the impairment of the circulation of the lung in pneumonia. A study of the distribution of the fibrin was made to determine how it produced the obstruction.

Microscopic examination of the pneumonic lungs from the above experiments showed a large number of fibrin plugs in the capillaries and even in the larger vessels. The only exception was the consolidated lung of the animal that had received chloroform and phosphorus. The fibrin thrombi have been observed by others (10, 11), but they were considered to be a postmortem, or at most an agonal, phenomenon and of no significance in the pneumonic process.

That they occur early in the course of pneumonia can be demonstrated from the experiment with trypan blue. This is a most readily diffusible dye and reaches all areas whose blood supply is not impaired. When pneumonia is produced in animals in whose circulation this dye is present, the consolidated area becomes uniformly stained. If the dye is injected twenty hours after the pneumonia is produced, the consolidated area is not uniformly stained, and, as has been said, it may show sharply demarcated and absolutely unstained areas.

It may, therefore, be concluded that fibrin plugs form in the capillaries early in pneumonia and that these impair the circulation in the consolidated lung. The distribution of the fibrin plugs in the ves-

<sup>1</sup> We are indebted to Drs. Whipple and Goodpasture for aid in this experiment.

sels in the pneumonic lung of man is relatively uniform throughout, and by their interference with the circulation they probably cause the pallor of the lung in gray hepatization. In the pneumonic lung of the rabbit the distribution of these plugs is not uniform; they may be so extensive that there is an absolute loss of circulation in local areas. Such areas are likely to undergo necrotization and probably result in the minute abscesses which have been found repeatedly in rabbits' lungs long after the pneumonic process has subsided.

The importance of this wide-spread formation of fibrin plugs in the vessels in the pneumonic lung is at once evident. The experiments of Opie (12) have shown the presence of an antitryptic substance in the serum which prevents the autolysis of leucocytes and the digestion of fibrin by leucocytes. The leucocytes alone digest this material rapidly. Therefore, with an interference of the circulation the leucocytes of the exudate are much less influenced by the action of the serum which reaches them in minimum amount, and as a result autolysis takes place in the exudate just as it would if the pneumonic lung were placed in the thermostat. There can be no doubt that this interference in the circulation in the pneumonic lung is of great importance in the resolution of the exudate.

It is further probable that the interference of the circulation in the pneumonic lung is responsible for the restricted action of immune serum in this disease. The experiments of Cole (13) have shown that the septicemia of pneumonia, and consequently the fatal outcome of the disease, may be averted in many cases by the intravenous administration of the specific immune serum, but the progress of the local lesion remains uninfluenced. This must be expected since, on account of the impaired circulation, the immune serum can not reach the local lesion in sufficient amounts to influence its progress. It is evident that this same criticism would apply to any therapeutic measure so administered, and it therefore seemed advisable to ascertain whether the consolidated area could be impregnated in some other way.

The following experiments were carried out to see whether the consolidated area could be stained by an intratracheal injection of a dye.

Pneumonia was produced in a rabbit, and forty-eight hours later, under ether anesthesia, the anterior thorax was removed, and artificial respiration by the Meltzer method of intratracheal insufflation was instituted. Respiration was interrupted long enough to allow the injection of 10 c.c. of a 1 per cent. solution of trypan blue into the consolidated lung through a catheter inserted into its bronchus. The animal was then killed by ether.

On section of the consolidated lung it was found to be uniformly and intensely stained. Microscopic section showed the presence of the dye in practically all the alveoli localized in the same elements which are stained after intravenous injection of the dye.

This experiment was repeated with a similar result. It may be said, therefore, that the presence of fibrin plugs throughout the capillary bed of the pneumonic lung interferes greatly with the penetration even of such a diffusible substance as trypan blue, when this drug is injected intravenously, but the exudate offers no serious obstruction to the penetration of the dye into the alveoli when it is injected intrabronchially.

The method of intrabronchial treatment of pneumonia therefore suggests itself.

#### CONCLUSIONS.

1. Dead leucocytes are constantly found in the pneumonic exudate. They rapidly undergo disintegration. Up to the seventh day they do not form the majority of the cells of the exudate.
2. Polymorphonuclear leucocytes with vitally stained granules are present in the exudate, vessels, and interstitial tissue of the lung in experimental pneumonia, but they are not demonstrable in the general circulation in the same animals.
3. There is a marked impairment of the circulation in the pneumonic lung.
4. The increase in the intra-alveolar pressure exerted by the exudate has no influence upon the circulation.
5. The impaired circulation results from the wide distribution of capillary fibrin thrombi. In man these are, as a rule, distributed with relative uniformity. In the rabbit this is not usually the case. The thrombi are much more abundant in some areas and may lead to localized necrosis.
6. The impairment of the circulation is of importance in bringing about resolution. Only enough blood is allowed to seep through



the vessels to nourish the alveolar walls. Consequently very little serum escapes into the alveoli and the autolysis of the exudate by the leucocytes is unhindered.

7. The impairment of the circulation in the pneumonic lung seriously interferes with the action of intravenous therapy upon the local lesion.

8. The exudate in the pneumonic lung can be readily impregnated with a dye injected intrabronchially. This suggests a method of administration of therapeutic agents in pneumonia.

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