Welch (Wm H.) & Flexner (S.)

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THE HISTOLOGICAL LESIONS PRODUCED BY THE TOX-ALBUMEN OF DIPHTHERIA.

BY

WM. H. WELCH, M. D., *Professor of Pathology*,

AND

SIMON FLEXNER, M. D., *Fellow in Pathology*.

In a preliminary communication presented to the Johns Hopkins Hospital Medical Society, and published in the HOSPITAL BULLETIN, No. 15, August, 1891, we called attention to the histological changes in the organs of animals which had died of experimental diphtheria, following the inoculation of pure cultures of the bacillus diphtheriæ. Since then we have extended our investigations so as to include the study of the lesions produced by the inoculation of the toxic products of the diphtheria bacillus. This study virtually finishes the work we have undertaken, and it is hoped soon to publish our results in detail. However, in order to make our preliminary communication complete, we append this report.

The toxic products of the diphtheria bacillus with which we have operated were obtained by filtering through a new and sterilized Chamberland filter a culture of the organisms in glycerine-bouillon several weeks old. The fluid so obtained was tested by means of cover-slips and inoculations on glycerine-agar, and proved to be sterile.

Guinea-pigs were used for the experimental inoculations. The sterile culture fluid was introduced subcutaneously into the tissues of the belly-wall. The method pursued will be given in connection with the case of which the lesions are to be described. This guinea-pig received on the 10th of December, 1891, 1 cc. of the filtrate. Not having succumbed, on December 14th it received 2 cc. more. The animal died on January 5th, 1892, the duration of life since the first inoculation having been three weeks and five days, and since the last, three weeks and one day.

At the autopsy the vessels of the subcutaneous tissues were

injected, and hemorrhage had taken place into the tissues of the axillary and inguinal regions. The subcutaneous tissues were moist, but there was no actual œdema present. Neither was there a visible area of localized inflammation. However, no microscopical examination was made. The lymphatic glands of the axillary and inguinal regions were enlarged and reddened; the cervical lymph glands were swollen, and the thyroid gland was greatly congested.

There was a considerable excess of clear fluid in the peritoneal cavity. Both layers of the peritoneum were reddened, the vessels of the visceral layer being especially injected. The spleen was enlarged to double the average size. It was mottled, and the white follicles were distinctly outlined against the red ground. The liver was dark in color and contained much blood. On the surface a prominent yellowish-white area 2 mm. in diameter, surrounded by a zone of hyperæmia, was observed. Smaller dot-like points of the same color and general appearance were seen elsewhere in the liver. The kidneys were congested and the cut surface was cloudy. The adrenal glands appeared normal, as did the mesenteric glands.

The pleural cavity did not contain such a marked excess of fluid. The pericardial sac, however, was distended with clear serum. Under the epicardium were many ecchymotic spots. The lungs exhibited areas of intense congestion, or actual hemorrhage into the tissues. The glands of the thorax were, perhaps, swollen.

The examination of frozen sections showed the heart muscle to be slightly fatty. The epithelium of the tubules of the kidney was extremely granular and much swollen, but not fatty. The liver was very fatty; the lighter areas and dots were seen to correspond to foci of dead liver cells, whose refraction was much greater than that of the normal cells.

Cultures were made from the blood and organs of the animal and they remained sterile. Cover-slips were also examined and no organisms found.

The histological lesions observed in this case are identical with those described by us in connection with the inoculation of the living organisms. Lymphatic apparatus: In general, the changes are the same throughout. They are found in the greatest intensity in the glands of the axillary and inguinal regions, and less in the bronchial, cervical, mediastinal and

mesenteric glands. Yet these are considerably affected. The same fragmentation of nuclei, affecting the lymph nodes and sinuses, is met with. These fragments exhibit the variety of form previously described by us, and they have the same affinity for coloring agents. Much of the nuclear detritus is free, but a part is contained within large pale cells. In the spleen there is a similar diffuse fragmentation of the nuclei of the spleen cells. Both the lymphoid cells of the follicles and the larger cells of the sinuses are affected. Like the lymphatic glands, some of the nuclear detritus is enclosed in large cells. Besides the destruction of cells in the spleen there is hemorrhage into the organ, or an extreme degree of congestion, so that the tissue elements are widely separated from one another. Nuclear figures occur in the lymph glands and spleen. In the former they are found among the fragmented cells.

Stained sections of the liver, especially those stained in methylene-blue and eosine, show the yellowish-white areas to be composed of hyaline, necrotic liver cells. The necrotic cells stain deeply in the eosine, and they are usually devoid of nuclei. They form, on the whole, more or less definite foci of hyaline cells into which leucocytes have wandered. The largest area was 2 mm. in diameter, and the outlines of it were formed by hemorrhage into the tissues, corresponding with the hyperæmic zone spoken of above. The cells in this focus have lost their nuclei and they are intensely refractive. Many of the dead cells have retained their individuality, and, indeed, their borders are more distinct than those of the normal cells. Others, however, tend to become fused together and to lose their individual cell outlines. Occasionally, outside the main focus of hyaline cells, single necrotic cells occur which are surrounded by quite normal ones. Many leucocytes have wandered into this area of dead cells, and they are especially abundant at one place in the focus in which the hyaline and necrotic cells are in process of disintegration. An exquisite nuclear fragmentation is to be observed throughout this area.

Should the focus just described be compared to many similar foci which occur in the livers of animals dead of inoculation with the bacilli themselves, it will be seen to contain more leucocytes (polynuclear) within it. The explanation of this fact would seem to depend somewhat on the incubation

time, but more on the progression or stage of the necrotic process. Inoculation of the bacilli usually leads to death in a very short time, often in 24 to 48 hours. In this inoculation with the toxic products alone, the incubation period exceeded three weeks. On account of this, time has been allowed for the softening and disintegration of the dead cells, and leucocytes have been strongly attracted to these foci.

In the kidneys, besides the condition described in the frozen sections, a slight fragmentation of the nuclei of the epithelium of the tubules is encountered. The lungs exhibit areas of hemorrhage into the alveoli, and in many of these there has been a desquamation of the alveolar epithelium. Sometimes the desquamated epithelial cells are quite normal in appearance, while at others they have fragmented nuclei. The collections of lymphoid cells around the medium-sized and larger bronchi show, however, more cells, the nuclei of which have suffered in this way.

The blood-vessels of the tissues generally contain fewer leucocytes in this instance than in those cases in which the bacilli were introduced beneath the skin. By the latter method an intense local inflammatory process is provoked, associated with the emigration of large numbers of polynuclear leucocytes. In the former, in which the filtrate, free from organisms, is used for inoculation, the local process is reduced to nil, there is no emigration of leucocytes, and the disease is general from its inception. This difference is sufficient to account for the occurrence of leucocytosis in the one case and its absence in the other.

It may be considered as established now that the toxic products and not the bacilli themselves invade the tissues in diphtheria. This fact would at once suggest that the general lesions (those produced at a distance from the seat of inoculation in animals, and the situation of the local process in human beings) were the effects of the soluble poison diffused through the body. Hence, it was desirable to demonstrate this assumption experimentally; and it is not unimportant to know that the lesions in the tissues produced by the bacilli and the toxic principle on the one hand, and the toxic principle alone on the other, are in perfect correspondence with each other. And, moreover, it would seem not to be superfluous to emphasize the occurrence of definite focal lesions in the tissues of the body, produced by a soluble poison circulating in the blood.