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THE SITE AND RATE OF DESTRUCTION OF PNEUMOCOCCI FOLLOWING INTRA-PERITONEAL INJECTION

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In a recent publication concerning the physiological destruction of erythrocytes, Kyes (1) has shown that certain endothelial cells of the liver and spleen are constantly active in phagocytosing red blood corpuscles from the circulating blood stream and to the fixed-tissue phagocytes so functioning, he gives the designation "hemophages."

In a subsequent communication concerning the natural immunity of the pigeon to the pneumococcus, the same author (2) points out that under experimental conditions, the hemophages are not only phagocytic for red blood corpuscles but also for pneumococci introduced into the circulating blood stream. Indeed, so rapid and so extensive is the phagocytic destruction of injected pneumococci by the hemophages of the pigeon, that Kyes concludes that this mode of elimination of the organisms from the blood stream is largely responsible for the high resistance which the pigeon displays to pneumococcus infection. The great importance of the hemophages in this immunity appears the more probable since Kyes observed that pneumococci introduced into the body cavities of the pigeon were rapidly transported to the blood stream and were then also eliminated by the hemophages after the same manner as those organisms injected directly into the blood stream.

The present study is a further analysis of the extent to which pneumococci introduced into the peritoneal cavity are destroyed by hemophages in the liver and spleen and is also a determination of the rate of the destruction of the bacteria so introduced in comparison with those injected directly into the blood stream.

The fate of inert, insoluble particles injected into the peritoneal cavity has been investigated by Muscatello (3). Employing a suspension of insoluble carmine, this worker found that after a period of from one and one-half to two hours, the pigment granules were present in the liver and spleen, either free within the blood vessels or within leucocytes of the blood stream. In smaller numbers, he also found the carmine granules in the lung, pancreas, and the testes. Maffuci (4) conducted similar experiments, and after the same lapse of time also found the pigment particles in the liver and spleen, but in those organs only. Maffuci decided also, that the position of the granules was the same in the liver whether the injections had been made into the peritoneal cavity or the jugular vein. Buxton and Torrey (5) in an extended series of experiments with lamp-black injected into guinea pigs, further confirmed the general findings of Muscatello. In studying also the distribution of typhoid bacilli injected intraperitoneally into rabbits, Buxton and Torrey showed by cultural methods, that the bacilli very rapidly reached the blood stream and were deposited in various organs, "the liver showing the greatest power of holding up to the organism." Strouse (6) on the other hand, working with intraperitoneal injections of pneumococci into pigeons did not find a distribution of the organisms to the liver or spleen and in but two pigeons of a series of fourteen did he recover the organisms from the heart's blood.

EXPERIMENTS

With the purpose of comparing the fate of pneumococci injected intravenously with that of those introduced intraperitoneally, we injected parallel series of pigeons by these two avenues with the same amounts of one and the same suspension of pneumococci and killing the animals after the lapse of various time intervals, determined the distribution of the organisms within the various tissues by systematic microscopic examination. Recognizing the unreliability of fine quantitative distinctions based upon the direct numerical count of bacteria in tissues, we have neglected minor differences and taken into consideration only contrasts of such degree as to be beyond the limits of error of observation and of interpretation. The pneu-

mococcus culture used was one freshly isolated from a case of acute lobar pneumonia in man. This organism was grown on blood agar slants in one litre flasks, the surface of each of which was approximately equivalent to that of twenty ordinary blood agar slants in test tubes. After incubating these flask cultures for twenty-four hours at 37°C. the total growth was suspended in such a quantity of physiological salt solution that two cc. of the suspension contained approximately the same number of pneumococci that would be obtained from two and one-half blood agar slants. For a single series twenty-two pigeons were used—eleven receiving 2 cc. of the above suspension intravenously, and eleven receiving 2 cc. intraperitoneally. These birds were killed after the lapse of 10 minutes, 30 minutes, 1, 2, 3, 6, 9, 12, 18, 24, and 36 hours following injection. Immediately after killing, thin pieces of the liver and the spleen from each bird were placed for 24 hours, in Zenker's solution without acetic acid. After imbedding in paraffin, sections 3 to 4 μ in thickness were cut and fixed on slides. The sections were further prepared by the method used by Kyes (2) in his study of pneumococci injected intravenously into pigeons. Briefly the method is as follows: To differentiate the hemophages the sections are first subjected to treatment with potassium ferrocyanide and hydrochloric acid. They are then stained with acid carmine and by Gram's method. A point of considerable importance in connection with the latter is the careful decolorization in a mixture of toluol and aniline oil, approximately in the proportion of two to one. The use of alcohol after Gram's stain decolorizes the specimen too rapidly and completely. With the technic mentioned, the Gram positive pneumococci when present, stand out prominently in the distinctly differentiated blue-green hemophages.

When the tissues of a complete series of birds were fixed as outlined above, each observer stained and examined a series of slides from each pigeon and tabulated his results independently. These results were then compared and gone over again where there was any difference of opinion, so that the combined results represent observations on two complete sets of slides from the same bird, each set being prepared and examined by a different

individual. As an additional control on the tissues which failed to show pneumococci in the double staining method, at least five slides from such tissue were stained with Gram's stain alone, before it was concluded that the organisms were not present.

Discussion of Results

The results obtained in the four different series were parallel and uniform except for slight individual variations which were inconsiderable from the point of view of the demonstration in question. The protocol of the single series here reported is illustrative therefore of the total four series investigated.

Stated in general terms, the results obtained by the methods outlined showed that following intraperitoneal as well as intravenous injection, the pneumococci are phagocytosed by hemophages in the liver and spleen and furthermore that the organisms so taken up are rapidly destroyed within the containing cell. Also, that the appearance of the pneumococci in the liver and spleen is later following intraperitoneal than intravenous injection but as extensive. Before discussing the results in detail however, the approximate quantitative results obtained may be charted as follows:

TABLE 1

TIME INTERVAL	CONCENTRATION OF ORGANISMS			
	Intraperitoneal		Intravenous	
	Liver	Spleen	Liver	Spleen
10 min.	0	0	+	+
30 min.	0	0	$+^{2}$	$+^{2}$
1 hr.	0	0	$+^{5}$	$+^{5}$
2 hrs.	+	+	$+^{8}$	$+^{7}$
3 hrs.	$+^{4}$	$+^{4}$	$+^{7}$	$+^{6}$
6 hrs.	$+^{8}$	$+^{8}$	$+^{2}$	$+^{2}$
9 hrs.	$+^{2}$	$+^{2}$	+	+
12 hrs.	+	+	+	+
18 hrs.	0*	0*	+	+
24 hrs.	0*	0	0*	0*
36 hrs.	0	0	0	0

* Possibly a few remnants of digested cocci, but no organisms of definite form or staining reaction.

The results tabulated above show that tremendous numbers of pneumococci were distributed to the liver and spleen not only when introduced intravenously but also when injected intraperitoneally. Furthermore the pneumococci within these organs were located for the most part within hemophages, irrespective of the mode of injection.[1]

The single point of difference in the distribution of the bacteria following the two modes of injection is to be observed in regard to the time of the localization of the organisms within the liver and spleen. Following intravenous injection of the bacteria, their partial localization in the liver and spleen is accomplished at the time of the earliest observation, namely ten minutes. With the intraperitoneal introduction on the other hand, the first evidence of such localization occurs at the two-hour observation. The same relation obtains also in regard to the period of maximum accumulation of the bacteria in these organs. Thus, the pneumococci were found in greatest numbers in the hemophages of the liver and spleen two and three hours after intravenous injection, whereas after intraperitoneal injection, the maximum bacterial content was found occurring distinctly later, namely from three to six hours after the injection.

The period of survival of the pneumococci after their accumulation within the liver and spleen cannot be determined with any great degree of accuracy. This is because it is impossible to identify absolutely the last remnants of the digested organisms and therefore to establish the exact time of the complete disappearance of bacterial remains. Our experiments do show however, that both the liver and spleen of birds injected intravenously show a much reduced content of organisms at the six hour period, a corresponding reduction appearing at the nine-hour period of birds injected intraperitoneally. At subsequent periods the number of organisms found was inconsiderable, only partially digested remnants being present after eighteen hours and even these being entirely absent at thirty-six hours. At

[1] The details as to localization will not be included here in as much as they coincide with the descriptions already given in extenso by Kyes in a communication previously referred to.

the twenty-four-hour period no intact, normally staining organisms were present, but it was impossible to determine whether or not occasional dark granules within the hemophages were end products of bacterial digestion. Without attempting therefore to establish the exact time of their complete disappearance, it may be stated that under the conditions of our experiments, the bulk of the pneumococci localized in the liver and spleen are destroyed within six hours after the first appearance of the bacteria in those organs.

CONCLUSIONS

The conclusions which may be drawn from the foregoing results are:

1. That pneumococci injected intraperitoneally into pigeons are rapidly transported to the liver and spleen and are there localized within fixed tissue phagocyes after exactly the same manner as are pneumococci introduced directly into the blood stream.

2. That, judged both from the time of the appearance of the first cocci and that of the maximum concentration of cocci in the liver and spleen, the period required for the transportation of the organisms from the peritoneal cavity to the circulating blood stream is approximately two hours.

3. That pneumococci which are localized in the liver and spleen following intraperitoneal injection are destroyed within twenty-four hours of their arrival in those organs.

This study was undertaken at the suggestion of Professor Preston Kyes and we desire to express our appreciation of his interest and assistance during the progress of the work.

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