STUDIES UPON EXPERIMENTAL PNEUMONIA IN RABBITS.

V. The Rôle of the Leucocyte in Experimental Pneumonia. The Relation of the Number of Organisms Injected to the Mortality.*

By B. S. KLINE, M.D., and M. C. WINTERNITZ, M.D.
(*From the Pathological Laboratory of Johns Hopkins University, Baltimore.*)

It has been definitely shown that lobar pneumonia may be produced in rabbits by the Meltzer method of intratracheal inoculation of cultures of pneumococci (1). The disease produced in this way runs a relatively rapid course, and, in the previous reports, it was noted that the animals died, as a rule, within a few days. It has been demonstrated further that in animals previously treated with benzol the pneumonia produced is much more rapidly fatal. Benzol, as is now well known, is a powerful leucotoxic substance not only destroying the leucocytes in the circulating blood but also affecting the bone marrow particularly, and the other hematopoietic organs to a less extent.

The rapidly fatal course of the pneumonia in animals in which the leucocytes had been previously destroyed to a great extent was further emphasized by a characteristic histological picture in the lungs of the animals. The pneumonic exudate consisted chiefly of fibrin and pneumococci, the latter in such large numbers that they densely stippled the entire section. These facts have naturally led us to associate the rapidly fatal outcome in these experiments with the diminished number of leucocytes. Before this association can be definitely established it is necessary to show that benzol aside from its action on the blood and hematopoietic organs does not lessen the resistance of the animals in some other way.

This question may be approached by the substitution of toluol

* Received for publication, April 16, 1913.

for benzol.[1] Toluol differs from benzol only in having an additional methane radicle.

Benzol C_6H_6 Toluol $C_6H_5CH_3$

Toluol repeatedly injected into rabbits in exactly the same manner as benzol (one cubic centimeter of toluol + one cubic centimeter of olive oil per kilo of body weight, subcutaneously) is followed by certain local and general effects. Local sloughs may occur, the animals usually lose weight and in their general appearance are not unlike the animals treated with benzol. They may die from often repeated doses.

The two drugs, however, differ markedly in their effect upon the blood and blood-forming organs. No demonstrable change in the leucocyte count is present even after many doses of toluol, as may be seen from table I.

The changes in the bone marrow are of interest in this connection, for, as we shall show, the repeated injection of toluol is followed by a definite hyperplasia of this tissue. Several animals received six daily doses of toluol subcutaneously (one cubic centimeter in one cubic centimeter of olive oil per kilo of body weight) and were killed on the seventh day. The histological picture of the hematopoietic organs may be briefly stated as follows: The most marked changes appear in the bone marrow. Those in the lymph glands and other blood-forming organs are so slight that they may be omitted. The bone marrow shows a distinct hyperplasia involving particularly the myeloid elements. The cellular proliferation is so marked that the fatty marrow is almost entirely obliterated.

[1] The metabolism by the animal body of toluol differs from that of benzol. Toluol is oxidized to benzoic acid (C_6H_5COOH) and then combined with the glycocoll of the body is excreted as hippuric acid ($C_6H_5CO \cdot NHCH_2COOH$). Benzol is oxidized to phenol (C_6H_5OH) and to dioxybenzols (C_6H_4OHOH) which combine in the kidney with sulphuric and glycuronic acids to form phenolsulphuric ($C_6H_5OSO_3H$) and phenolglycuronic acids and the corresponding dioxybenzol compounds (Cushny, A. R., A Textbook of Pharmacology and Therapeutics, or the Action of Drugs in Health and Disease, 5th edition, Philadelphia, 1910, 448).

TABLE I

Leucocyte Counts Before and After Injection of Toluol.

No. of animal.	Weight in gm.	Duration of experiment.	No. of injections of toluol.	White blood count before toluol.	White blood count after toluol.
3 A	1,200	8 days	6	8,200	8,400
3 B	1,570	8 days	6	8,800	9,700
2 A	1,750	8 days	6	9,000	9,900
2 B	1,870	8 days	6	10,500	14,000
46	1,130	8 days	5	7,600	13,000
59	1,720	7 days	4	7,000	5,700
60	1,580	7 days	3	7,800	5,800
61	1,850	7 days	6	16,900	13,400
62	1,360	7 days	5	8,900	6,900
63	1,290	7 days	6	15,700	13,200

The fact that following the repeated injections of toluol there is a definite hyperplasia of the bone marrow without an accompanying leucocytosis corroborates the impression gained in the work with benzol that the blood picture is not always an absolute index of the condition of the marrow.

With these preliminary tests in mind, several series of experiments, of which the following is an example, were performed.

Thirteen rabbits varying in weight from 1,300 to 1,900 gm. were used. Five were treated with repeated doses of benzol (three to six subcutaneous injections of 1 c.c. in an equal quantity of olive oil per kilo of body weight) until the circulating white blood cells were reduced, with but one exception, to between 420 and 720 per cubic millimeter. In one animal the cells still numbered 2,800 per cubic millimeter after six injections of benzol. Four animals were injected in a similar manner with toluol (three to six subcutaneous injections of 1 c.c. in an equal quantity of olive oil per kilo of body weight). The toluol and benzol groups of animals corresponded as nearly as possible in weight, nutrition, and general appearance.

Four control rabbits, corresponding likewise in weight and general appearance to four animals in each of the preceding groups, were included in the series. After the preliminary treatment with benzol and toluol all the animals including the controls were inoculated intratracheally with a culture of pneumococci.

The results of the experiment are presented in table II.

TABLE II.

Date.	Benzol.			Toluol.			Control.		
	No. of animal.	White blood count.	Remarks.	No. of animal.	White blood count.	Remarks.	No. of animal.	White blood count.	Remarks.
Dec. 12, 1912	56	18,000	Weight 1,550 gm.	60	7,800	Weight 1,580 gm.	66		Weight 1,390 gm.
Dec. 13			1.55 c.c. benzol subcutaneously			1.58 c.c. toluol subcutaneously			
Dec. 14			1.55 c.c. benzol subcutaneously			1.58 c.c. toluol subcutaneously			
Dec. 15			1.55 c.c. benzol subcutaneously			1.58 c.c. toluol subcutaneously			
Dec. 16		800							
Dec. 17		260							
Dec. 18		480							
Dec. 19		440	5 c.c. of 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.		5,800	5 c.c. of 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.		5,400	5 c.c. of 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.
Dec. 20									
Dec. 21			Found dead 41 hrs. after injection. Lobar consolidation of left upper, $\frac{3}{4}$ of left lower lobe. Blood culture positive		11,000			10,000	
Dec. 22					14,000			13,400	
Feb. 15, 1913						Killed 58 days after injection			
March 7									Well 79 days after injection.

TABLE II.—*Continued.*

Date.	Benzol.			Toluol.			Control.		
	No. of animal.	White blood count.	Remarks.	No. of animal.	White blood count.	Remarks.	No. of animal.	White blood count.	Remarks.
Dec. 12, 1912	58	7,400	Weight 1,360 gm.	62	8,900	Weight 1,360 gm.	64		Weight 1,340 gm.
Dec. 13			1.36 c.c. benzol subcutaneously			1.36 c.c. toluol subcutaneously			
Dec. 14			1.36 c.c. benzol subcutaneously			1.36 c.c. toluol subcutaneously			
Dec. 15			1.36 c.c. benzol subcutaneously			1.36 c.c. toluol subcutaneously			
Dec. 16		4,000	1.36 c.c. benzol subcutaneously			1.36 c.c. toluol subcutaneously			
Dec. 17		1,300	1.36 c.c. benzol subcutaneously			1.36 c.c. toluol subcutaneously			
Dec. 18		760							
Dec. 19		420	5 c.c. of 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.		6,900	5 c.c. of 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.		11,500	5 c.c. of 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.
Dec. 20		380							
Dec. 21					16,100			14,900	
Dec. 22		860			17,900			12,900	
Dec. 23		260							
Dec. 24			Found dead 113½ hrs. after injection						
Jan. 24, 1913			Patchy consolidation of right lungs, most marked in the right lower. Beginning pleurisy. Blood culture positive						Killed 36 days after injection.
Mar. 7						Well 79 days after injection			

Date.	Benzol.			Toluol.			Control.		
	No. of animal.	White blood count.	Remarks.	No. of animal.	White blood count.	Remarks.	No. of animal.	White blood count.	Remarks.
Dec. 12, 1912	54	7,600	Weight 1,900 gm.	61	16,900	Weight 1,850 gm.	67		Weight 1,820 gm.
Dec. 13			1.9 c.c. benzol subcutaneously			1.85 c.c. toluol subcutaneously			
Dec. 14			1.9 c.c. benzol subcutaneously			1.85 c.c. toluol subcutaneously			
Dec. 15			1.9 c.c. benzol subcutaneously			1.85 c.c. toluol subcutaneously			
Dec. 16		4,800	1.9 c.c. benzol subcutaneously			1.85 c.c. toluol subcutaneously			
Dec. 17		4,200	1.9 c.c. benzol subcutaneously			1.85 c.c. toluol subcutaneously			
Dec. 18		5,000	1.9 c.c. benzol subcutaneously			1.85 c.c. toluol subcutaneously			
Dec. 19		2,800	5 c.c. of 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.		13,400	5 c.c. of 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.		6,600	5 c.c. of 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.
Dec. 20					20,500			6,500	
Dec. 21		400			15,600			6,100	
Dec. 22		500						13,700	
Dec. 23		100	Died 92 hrs. after injection. Lobar consolidation $\frac{5}{6}$ of right lower and $\frac{1}{2}$ of right middle lobe. Fibrinous pleurisy. Blood culture positive						
Jan. 3, 1913						Died 14$\frac{1}{2}$ days after injection. Areas of organized pneumonia and pleurisy in right lower lobe. Fibrinopurulent pericarditis. Blood culture positive			
Jan. 4									Died 16 days after injection. Extreme shaggy fibrinopurulent pleurisy and pericarditis, subdiaphragmatic abscess. Old lobar consolidation of right lower lobe. Blood culture negative.

TABLE II.—*Continued.*

Date.	Benzol.			Toluol.			Control.		
	No. of animal.	White blood count.	Remarks.	No. of animal.	White blood count.	Remarks.	No. of animal.	White blood count.	Remarks.
Dec. 12, 1912	55	15,800	Weight 1,560 gm.	59	7,000	Weight 1,720 gm.	65		Weight 1,550 gm.
Dec. 13			1.56 c.c. benzol subcutaneously			1.72 c.c. toluol subcutaneously			
Dec. 14			1.56 c.c. benzol subcutaneously			1.72 c.c. toluol subcutaneously			
Dec. 15			1.56 c.c. benzol subcutaneously			1.72 c.c. toluol subcutaneously			
Dec. 16		1,900	1.56 c.c. benzol subcutaneously			1.72 c.c. toluol subcutaneously			
Dec. 17		760							
Dec. 18		360							
Dec. 19		400	5 c.c. of 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.		5,700	5 c.c. of 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.		5,800	5 c.c. 20 hr. culture of pneumococcus intratracheally, 100,000 per c.mm.
Dec. 20					13,300				Died 28 hrs. after injection. Early lobar consolidation of right lung (3 lobes). Fibrinopurulent pleurisy and mediastinitis. Blood culture positive.
Dec. 21		260							
Dec. 22			Found dead 65 hrs. after injection. Lobar consolidation of right upper and middle lobes, central lobe, $\frac{3}{4}$ of right lower, $\frac{2}{3}$ of left upper with fibrinopurulent pleurisy. Blood culture positive		13,500				
Dec. 23									
Dec. 24						Found dead $113\frac{1}{2}$ hrs. after injection. Lobar consolidation of left upper, fibrinopurulent mediastinitis. Blood culture positive			

TABLE II.—*Continued.*

Date.	Benzol.		
	No. of animal.	White blood count.	Remarks.
Dec. 12, 1912	57	15,200	Weight 1,330 gm.
Dec. 13			1.33 c.c. benzol subcutaneously.
Dec. 14			1.33 c.c. benzol subcutaneously.
Dec. 15			1.33 c.c. benzol subcutaneously.
Dec. 16		5,500	1.33 c.c. benzol subcutaneously.
Dec. 17		3,200	1.33 c.c. benzol subcutaneously.
Dec. 18		1,240	1.33 c.c. benzol subcutaneously.
Dec. 19		720	
Dec. 20		380	
Dec. 21		360	
Dec. 22			
Dec. 23		560	
Dec. 24			Died 115 hrs. after injection. Lobar consolidation of central lobe with marked fibrinous pleurisy. Blood culture positive.

Analysis of Table II.—The first animal died twenty-eight hours after injection, and was a control. Occasionally during intratracheal inoculation definite injury is done to the larynx and trachea. Some of the animals die immediately of hemorrhage, others develop a septicemia which is fatal within twenty-four to thirty hours. It is possible that this may account for the rapid course of the infection in this animal. Following this five benzol rabbits died in rapid succession in 41, 65, 92, 113½, and 115 hours respectively after inoculation, averaging 85 hours. One toluol rabbit died 113½ hours after inoculation. The remaining three toluol and three control animals lived through the acute period of the disease, but one toluol rabbit died on the fifteenth day with a marked organizing fibrinopurulent pericarditis, and a control rabbit died on the sixteenth day with extreme shaggy organizing fibrinopurulent pleurisy and pericarditis. The similarity of the results in the toluol and control groups and their contrast to the results in the

benzol group are striking. Correspondingly the leucocyte counts in the control and toluol groups are in marked contrast to the counts in the benzol group. In the latter the counts were very low, while in the control and toluol animals the counts were either normal or increased.

These facts seem to indicate that if benzol and toluol are at all comparable, the lowered resistance of benzol animals to experimental pneumonia is dependent upon the action which benzol has upon the blood and blood-forming organs and not upon any other action it may exert.

Another important point concerning the relation of the leucocytes to the resistance of animals against pneumococcus infection is brought out by table II. It may be seen that all the animals receiving toluol responded to the inoculation of pneumococci with a leucocytosis. Not only was this leucocytosis present in all the toluol animals, but in those surviving the pneumonia the increase in the number of white cells was greatest, increasing from 5,800 to 14,000 and from 6,900 to 17,900. The results suggested the advisability of producing a leucocytosis in animals before the intratracheal inoculation of pneumococci to see whether or not the resistance of the animals to the infection is thereby increased. With the purpose of producing an artificial leucocytosis a large number of drugs were tried, but our experiments are still too unsatisfactory for a detailed report. However, it has been found that a leucocytosis can be produced in rabbits by repeated intravenous injection of nutrose (sodium caseinate), a protein salt compound first studied in this connection by Kier (2).

In a preliminary experiment the few animals treated with nutrose before inoculation with pneumococcus culture gave analogous results to those treated with toluol. While the experiments are still too meager to draw absolute deductions, the following series has warranted further investigation. Three control rabbits and two in which the leucocytes were increased from 10,700 to 26,700 and from 11,600 to 28,000, by repeated intravenous injections of nutrose were each inoculated intratracheally with five cubic centimeters of a twenty-five hour stock culture of pneumococcus

in pig serum broth containing approximately 375,000 organisms per cubic millimeter. The three controls died in 56 hours, 5 days, and 10 days, respectively. One nutrose animal died in 67 hours and the other is still living 47 days after injection.

There are still many questions to be answered relating to the rapidly fatal course of experimental pneumonia in animals rendered aplastic with benzol. Perhaps the most important one is whether or not the resistance of these rabbits may be increased by the intravenous injections of leucocyte suspensions. At any rate, these preliminary experiments in which it is shown that rabbits with low white blood counts are more susceptible than those with normal counts emphasize the importance of the rôle of the leucocyte in experimental pneumonia.

A third point of interest is also brought out by the table. It will be remembered that in previously reported studies upon experimental pneumonia in rabbits the disease ran a rapidly fatal course in the large percentage of cases and only in a very few was death delayed for a longer period of time. In this series, on the other hand, and throughout more recent experiments a considerable number of animals have survived the intratracheal inoculation of pneumococci. This has been of particular interest since the same culture has been used in all but a few instances, in which other strains were used to determine if these might also produce the characteristic picture. Experiments were conducted in the hope of determining the cause of this difference in mortality.

In one series twelve small rabbits were used. All were inoculated intratracheally with a stock culture of pneumococci in pig serum broth. In determining the number of bacteria each pair was counted as one organism. Of the three animals each receiving 5 c.c. containing approximately 33,000 organisms per cubic millimeter, two survived the infection. Of the three each receiving 5 c.c. containing approximately 50,000 per cubic millimeter, one survived the infection. The two animals each receiving 5 c.c. containing 100,000 organisms per cubic millimeter, the two each receiving 5 c.c. containing 130,000 per cubic millimeter, and the two each receiving 200,000 per cubic millimeter, all succumbed to the infection. In another series, as noted above, three animals receiving 5 c.c. with approximately 375,000 organisms per cubic millimeter all died, and in still another series all four animals receiving 5 c.c. with approximately 500,000 per cubic millimeter succumbed to the infection. On the other hand, of eight large animals weighing 1,710 to 2,780 gm., all recovered from the intratracheal inoculation of 5 c.c. of stock culture of pneumococci in pig serum broth containing 25,000

organisms per cubic millimeter in four instances, and 17,000 per cubic millimeter in the remaining four.

The experiments indicate definitely that the number of pneumococci injected have an important bearing upon the mortality in the inoculated rabbits, many or all of those receiving small doses recovering and many or all receiving larger doses succumbing.

From subsequent experiments we have been led to the belief that while the number of organisms injected is of great importance in determining the mortality it is not the only factor. Further work is being done to determine more definitely the other influences.

CONCLUSIONS.

1. The importance of the leucocyte in the resistance of animals to experimental pneumonia is emphasized by the fact that animals treated with benzol, a leucotoxic substance, rapidly succumb to the disease, while animals treated in like manner with toluol, a very similar chemical substance causing no leucopenia, show no decreased resistance.

2. The rôle of the leucocyte in the resistance of animals to experimental pneumonia is further emphasized by the fact that animals that respond to the pneumococcus infection with a leucocytosis, as occurs after the repeated injection of toluol, are more resistant to the pneumonia. Further, the hyperleucocytosis produced by repeated injection of nutrose before the production of pneumonia likewise seems to increase the resistance of the animals.

3. Experimental pneumonia is not necessarily fatal in rabbits. The factors determining the outcome of the disease are numerous; among these is the number of bacteria inoculated. Animals receiving small doses usually survive, while those receiving comparatively large numbers usually succumb.

BIBLIOGRAPHY.

1. Winternitz, M. C., and Hirschfelder, A. D., *Jour. Exper. Med.*, 1913, xvii, 657. Hirschfelder, A. D., and Winternitz, M. C., *ibid.*, 666.
2. Kier, *Nord. Med. Ark.*, 1905, xxxviii, 1.