THE PASSAGE OF TYPE III RABBIT VIRULENT PNEUMOCOCCI FROM THE VASCULAR SYSTEM INTO JOINTS AND CERTAIN OTHER BODY CAVITIES*†

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It is well known that in certain bacterial infections characterized by blood stream invasion there occur, with varying frequency, instances of articular involvement which may progress to pyogenic arthritis. Additional information concerning the ease with which various pathogenic microorganisms gain entrance into the joint in comparison with other body cavities, and the manner by which invasion takes place, is necessary in order to obtain a better understanding of the specific infectious arthritides of man. An opportunity to make such observations in the case of one type of bacterial agent presented itself during the course of study of experimental infections with rabbit virulent strains of pneumococcus Type III. In these experiments the entrance of these non-motile, encapsulated organisms from the vascular system into joints was investigated. In addition, in the majority of animals, the aqueous humor, spinal fluid, and urine were examined. The effect of administration of specific antiserum on the joint infection was also investigated in certain experiments. The results obtained form the basis of this paper.

Methods

Normal albino rabbits, weighing from 3½ to 5 pounds, were employed throughout. Most animals were infected with the widely used SV strain of pneumococcus Type III. A few animals were inoculated (see Table II) with other rabbit virulent strains, the properties of which have been described elsewhere (1). Studies pertaining to the factors concerned in the rabbit virulence of these strains have been published (1–6).

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The cultures were grown at 37°C. in 7 cc. of meat infusion broth to which a drop of defibrinated rabbit blood had been added (designated as medium BB in the tables), or in a medium of 0.1 per cent dextrose, 0.5 per cent normal rabbit serum broth (designated as medium DSB in tables). These cultures, varying in age from 6 to 24 hours, were injected into a marginal ear vein in dosage of 0.05 to 2.0 cc.

At varying intervals the animals were sacrificed by rapid exsanguination, unless they had succumbed to the infection. Immediately thereafter specimens of synovial fluid, aqueous humor, spinal fluid, and urine were obtained in the order mentioned. The collection of these specimens was carried out in the following manner. The periarticular tissues medial to the infrapatellar ligament were cauterized and a small hypodermic needle, attached to a tight fitting syringe containing 0.5 cc. of sterile infusion broth, was inserted through the cauterized tissue into the joint beneath the patella. The broth was injected and withdrawn before removing the needle. This operation was facilitated by lifting the patella forward by means of traction on the infrapatellar ligament. Aqueous humor was obtained undiluted by aspiration of the anterior chambers with the aid of small hypodermic needles passed obliquely through the non-vascular cornea, after the latter had been sterilized with ethyl alcohol. Spinal fluid was aspirated from the cisterna magna after removing most of the overlying soft tissue and cauterizing those tissue layers that remained. Samples of urine were also taken by aspiration through a cauterized area of the bladder surface.

The synovial fluid washings and other body fluids thus obtained were employed as follows: 5 drops were streaked on a blood agar plate for culture, one drop was used for making glass slide smears, and the remainder was centrifuged to remove the cells and pneumococci and then used in certain cases as antigen for precipitative tests.

The air-dried, Wright-stained smears were examined for erythrocytes, nucleated cells, and pneumococci, thereby enabling us to determine whether or not the fluids were contaminated with blood or were inflammatory in nature.

In most of the experiments articular tissues were taken for microscopic examination. Such tissue specimens were fixed in Zenker's fluid, embedded and sectioned in paraffin, and stained by the Giemsa method. The information thus obtained was correlated with the other findings.

Two lots of antipneumococcus Type III serum were employed.[1] Both sera contained considerable type-specific precipitin and agglutinin and were effective in bringing about temporary sterilization of the blood stream infections. These sera were warmed before use and injected slowly, without undue pressure, into a marginal ear vein. The tests for the presence in the body fluids of serologically reactive substances derived from the antipneumococcal horse serum were carried out by means of the interfacial ring technique, employing the serum of a rabbit immunized against normal horse serum.

RESULTS

From Table I it will be seen that the cultures of the synovial fluid washings, aqueous humor, spinal fluid, and urine of 5 of the 7 rabbits examined 6 to 15½ hours following infection with the SV strain of Type III pneumo-

[1] One was obtained from the New York State Department of Health, and the other through the courtesy of Dr. Annabel Walters of the New York City Department of Health.

TABLE I

Results of Cultures from Certain Body Fluids in Rabbits Infected with Pneumococcus Type III Strain SV

Rabbit No.	Infecting dose	Period of infection	Number of organisms per cc. circulating blood at death	Cultures* and cytology† of body fluids									Microscopic changes in synovial membrane	
				Knee joint washings				Aqueous humor		Spinal fluid		Urine		
				Right		Left		Cultures						
				Culture	Smear	Culture	Smear	Right	Left	Culture	Smear	Culture	Right	Left
	cc.	hrs.												
3-7	16 hr. BB 0.2	6	60	0	0	0	0	0	0	0	0	0	0	0
4-8	16 hr. BB 0.2	7	80	0	0	0	0	0	0	0	0	0	0	0
1-06	16 hr. BB 0.2	$11\frac{1}{2}$	118,000	0	0	0	0	0	0	0	0	0	–	–
1-12	7 hr. BB 0.25	12	30,000	0	0	0	0	0	0	0	0	0	0	0
1-14	7 hr. BB 0.25	13	340,000	2	0	41	0	0	0	6	0 R.B.C.	0	0	0
1-18	22 hr. BB 0.1	$15\frac{1}{2}$	3300	0	0	0	0	0	0	0	0	0	0	0
4-3	7 hr. DSB 0.1	$15\frac{1}{2}$	38,000,000	115	–	30	–	0	0	0	–	0	0	0
4-4	7 hr. DSB 0.1	16	1200	0	–	++++	–	0	0	0	–	0	0	+
1-09	20 hr. BB 0.1	16	9000	0	+	0	0	0	0	0	0	0	+	0
1-22	22 hr. BB 0.1	16	21,000	++++	++++	C	++++	C	C	C	0	C	++	++
1-16	22 hr. BB 0.1	16	2400	0	0	0	0	0	0	0	0	0	0	0
5-1	24 hr. BB 0.1	$16\frac{1}{2}$	16,000,000	4	++	1	0	0	0	0	0	0	0	0
1-26	20 hr. BB 0.1	17	42,000	0	0	0	0	0	0	0	0	0	0	0
1-28	20 hr. BB 0.1	$17\frac{1}{6}$	50,000	0	0	++++	+++	0	0	0	0	0	0	0
4-9	24 hr. BB 0.1	$17\frac{1}{4}$	128,000	++++	++++	++++	++++	0	0	0	0	0	+++	+++
3-07	22 hr. BB 0.1	$23\frac{3}{4}$	33,000	++++	++++	8	+	0	0	–	0	–	+++	0
5-8	21 hr. BB 0.2	24	200,000	4	0 R.B.C.	0	0	0	0	0	–	–	0	0
3-3	21 hr. BB 0.1	$24\frac{1}{4}$	10,000	++++	++++	++++	++++	0	0	0	0	0	++++	++++
1-31	21 hr. BB 0.1	$29\frac{2}{3}$	–	++	++++	++++	++++	–	–	0	0	0	+	+
4-0	23 hr. BB 0.1	<40	++++	++++	0	++++	++++	–	–	–	–	–	–	–
1-40	10 hr. BB 0.1	<46	++++	++++	++++	++++	++++	–	–	–	–	–	–	–
10-00	24 hr. BB 0.1	52	++++	++++	–	++++	–	–	–	–	–	–	–	–
2-8	17 hr. BB 0.1	51	++++	++++	++++	0	0	++++	++++	++++	++	++++	+++	0
1-47	24 hr. BB 0.05	<135	++++	25	++++	0	++++	–	–	–	–	–	+++	++++

* The figures given in the underlying columns (culture) represent the numbers of colonies developing from 5 drops of fluid or joint washing plated on blood agar.

++ = several hundred colonies; ++++ = innumerable colonies producing a confluent growth. C = contaminated.

† Cytological changes as graded after examination of the stained smears are indicated in the underlying (smear) columns.

coccus were sterile. In 2 animals (Nos. 1-14 and 4-3) a few pneumococci were isolated from the joint washings, possibly representing minimal contamination with heavily infected blood. In favor of this interpretation is the absence of demonstrable cytological abnormalities in the synovial fluid smears of rabbit 1-14 and normal appearing synovial tissues from the knees of both animals.

Among 8 animals infected 16 to 18 hours, the synovial fluid washings of one or both knees of 4 contained large numbers of pneumococci. Moreover, infected joint fluids were obtained in 7 of the 8 animals infected 23 to 52 hours. In neither of these two groups did the occurrence of joint infection bear any direct relation to the number of organisms in the blood stream at the termination of the experiment. Thus, 2 rabbits (Nos. 4-4 and 1-22) infected 16 hours previously, showing terminal blood culture counts of 1200 and 21,000 pneumococci per cc. respectively, were found to have heavily infected synovial fluids in both knees, while in rabbit 5-8, infected 24 hours, having a terminal count of 200,000 viable cocci per cc., the synovial fluid cultures were sterile.

It is of interest to observe that in only 1 of 15 animals infected less than 18 hours was there unmistakable evidence of infection of both knees, whereas bilateral joint involvement was present in 6 of the 9 animals infected 23 hours or longer. It should also be noted that the aqueous humor, spinal fluid, and urine cultures were sterile in all 19 animals examined after periods up to 30 hours following inoculation. Unfortunately cultures of these body fluids were not obtained from the animals sacrificed at later periods except in one instance (rabbit 2-8), sacrificed at the end of 51 hours when moribund. In this instance of overwhelming infection, pneumococci had gained entrance into the aqueous humor of both eyes, the spinal fluid, and urine, yet only one knee was infected.

The study of the stained smears proved of great value in eliminating contamination of the joint fluid with blood as a possible source of error, since they were usually found to be in agreement with the cultural results. A sufficiently marked correlation also existed between the cytological abnormalities seen in the smears and the results of the cultures to enable one to predict in most instances that the culture would or would not be positive. In animals with blood stream infections of less than 16 hours' duration, having negative synovial fluid cultures, the stained smears from these same joint fluids showed relatively few nucleated cells, less than 10 per cent polymorphonuclear leukocytes, and very few or no red blood cells. The stained smears from heavily infected joint fluids always contained large numbers of nucleated cells, nearly all being polymorphonuclear

leukocytes, and in many instances morphologically distinguishable diplococci. The synovial fluid washings in such cases were often turbid in the gross and the synovial tissues showed microscopic evidence of acute inflammation.

A few discrepancies were recorded. The smear of the right knee joint washing of rabbit 4-0, which succumbed in less than 40 hours, showed no cytological abnormalities, yet the culture yielded innumerable colonies of pneumococci. Unfortunately no articular tissues were saved for microscopic examination. A similar discrepancy was noted in rabbit 1-47, dying on the 6th day. In this animal a few pneumococci were cultured from the right knee, the left knee joint washings being sterile, whereas the smears of both knees revealed the cytological alterations of acute inflammation. The gross and microscopic examination of these joint tissues gave evidence of acute inflammation, more marked in those from the left knee. In this instance, it is probable that both knee joints had been infected but with the development of antibodies and general immunity, the joint infections were overcome leaving residual evidence of inflammation.

TABLE II

Results of Heart's Blood and Joint Cultures from Rabbits Which Succumbed to Infection with Strains of Pneumococcus Type III Other than the SV Strain

Rabbit No.	Infecting strain	Culture dose	Period of infection	Number of organisms per cc. circulating blood at death	Cultures of knee joint washings	
					Right	Left
		cc.	*hrs.*			
7-0	C-Sp	1	40	++++	++++*	++++*
2-2	C-Sp	0.1	114	++++	41	−
2-3	IE	0.1	93½	356,000,000	0	++++*
2-80	B	1	129	++++	0	0
4-74	B	0.1	192†	0	++++*	0

* Synovial membrane shows fibrinopurulent exudate in the gross and microscopically.
† Animal died with fibrinous pericarditis and pleurisy.

That the results with the SV strain were not due to special properties of this particular organism is clearly shown in the data presented in Table II. Here one notes that similar knee joint infections occurred in rabbits infected with three other rabbit virulent strains of Type III pneumococci. Further examination of Table II reveals that the incidence of joint infections does not bear any direct relation to the number of organisms in the blood stream at the time the experiment was terminated. In rabbit 4-74 one knee was heavily infected, yet the blood culture was sterile; whereas rabbit 2-80, dying from an overwhelming infection from the same strain of pneumococcus had sterile knee joint washings.

TABLE III

Results of Cultures and Precipitinogen Tests with Certain Body Fluids from Rabbits Infected with Pneumococcus Type III Strain SV and Treated with Type-Specific Horse Antiserum

Rabbit No.	Infecting dose		Interval elapsing before serum treatment	Dose of antiserum administered intravenously	Interval elapsing after serum treatment	Cultures, precipitinogen tests, and cytology														
						Knee joint washings						Aqueous humor				Spinal fluid			Urine	
						Right			Left			Right		Left						
						Culture	Ppt.	Smear	Culture	Ppt.	Smear	Culture	Ppt.	Culture	Ppt.	Culture	Ppt.	Smear	Culture	Ppt.
		cc.	*hrs.*	*cc.*	*hrs.*															
5-4	8 hr. BB	2	$15\frac{1}{2}$	15	5	75	0	0	++++	0	0	−	−	−	−	−	0	0	−	−
3-6	8 hr. DSB	0.1	$16\frac{3}{4}$	10	$7\frac{1}{4}$	0	1/1	0	0	0	0	−	−	0	1/1	0	0	0	0	0
8-4	23 hr. BB	0.1	$17\frac{1}{3}$	9	8	0	0	0	0	0	0 R.B.C.	−	−	−	−	−	−	−	−	−
3-4	24 hr. BB	0.1	$17\frac{1}{2}$	10	$7\frac{1}{2}$	++++	−	+++	0	−	0	−	−	−	−	−	−	−	−	−
2-7	23 hr. BB	0.1	18	10	7	0	0	0	++++	1/1	+++	−	−	−	−	−	−	−	−	−
4	16 hr. BB	0.2	$18\frac{1}{2}$	15	7	0	0	0	++++	1/5	++	0	−	0	−	0	0	−	−	−
4-2	23 hr. BB	0.1	$24\frac{1}{6}$	10*	$24\frac{1}{2}$	++++	−	++++	++++	−	++++	++++	−	++++	−	−	−	−	−	−
3-5	24 hr. BB	0.1	30	10	$18\frac{1}{2}$	++++	1/1	++++	++++	1/1	++++	0	>1/1	0	0	0	0	0	−	−
3-9	21 hr. BB	0.1	33	7.5	19	0	−	0	++++	−	+++	0	−	0	−	0	−	0	0	−

* Administered intraperitoneally.

A number of rabbits infected with the SV strain were subsequently treated with a single intravenous or intraperitoneal injection of anti-pneumococcus Type III horse serum. The animals were sacrificed at varying intervals of time in order to determine whether the specific antiserum had been effective in either reducing the incidence of joint infection or eliminating the organisms from infected joints. An attempt was made also to learn whether the serologically reactive horse serum constituents had gained entrance into infected and non-infected body cavities. The results of these experiments are presented in Table III.

Rabbits 3-6 and 8-4 were infected 16¾ and 17⅓ hours before receiving antiserum. The joint washings were sterile when the animals were sacrificed 7 and 8 hours later. These results are inconclusive because, as has been shown in the previous experiments, joint infections do not regularly occur in animals infected for only 18 hours. The administration of specific antiserum at about this time probably resulted in at least temporary sterilization of the blood stream, thereby minimizing the likelihood of joint invasion during the subsequent survival period of 7 and 8 hours. The right knee joint washing and the aqueous humor of the left eye of rabbit 3-6 gave definite precipitative reactions with anti-horse rabbit serum. Thus it is clear that constituents of the horse serum had gained entrance into these body fluids. In rabbit 5-4, the cultures of the knee joint washings were positive, but the precipitative reactions with anti-horse rabbit serum were negative. The smears of the joint washings and microscopic sections of the synovial tissues revealed no evidence of inflammatory reaction, indicating that the bacterial invasion of the joint cavities was of short duration. The results in rabbits 2-7 and 4 are of considerable interest in that they suggest that the horse serum protein constituents enter inflamed joints more readily than the non-inflamed. Thus it will be seen that the infected joint washings of these two animals gave positive reactions with anti-horse rabbit serum, whereas the non-infected washings did not. Rabbit 4-2 received 10 cc. of antiserum intraperitoneally 24 hours after infection, yet both knee joints and one or both eyes (the aqueous humor from these two eyes was pooled) were infected when the animal was sacrificed 24 hours later. Rabbit 3-9 was given antiserum intravenously 33 hours after infection and sacrificed 19 hours later. In this animal the left knee joint was infected. Rabbit 3-5 received antiserum 30 hours after infection. When sacrificed 18 hours later, both knee joints were infected although horse serum constituents were present in the joint washings and the aqueous humor of the right eye.

DISCUSSION

Pneumococcal arthritis in man is a relatively rare disease (7). Chickering (8) has estimated the incidence of this complication of pneumococcal pneumonia as being approximately one-tenth of one per cent. A very occasional case is encountered without symptoms or signs of pulmonary involvement.

The incidence of articular localization of pneumococci following experimental infection of animals has not been established. Meyer (9) observed that articular involvement was rarely encountered in rabbits surviving at least 3 days following experimental infection with small doses of virulent pneumococci. Rosenow (10) noted joint involve-

ment in only one of 13 rabbits inoculated with pneumococci, which from his description must be regarded as atypical or modified strains. Davis (11) tested in rabbits 9 strains of organisms which he considered to be pneumococci. One animal developed a monoarticular arthritis. These studies were made before knowledge of the cultural dissociation and serological types of the pneumococcal group had been obtained. Davis concluded that true pneumococci rarely produced arthritis, whereas the injection of small doses of any one of six strains of rabbit virulent *Streptococcus mucosus* (his description agrees with that of the organism now generally called pneumococcus Type III) always resulted in involvement of articular structures (12). He warned that such highly virulent strains should be given in minute doses, otherwise the animals would die within 24 to 48 hours without definite localization of the bacteria.

The present experiments confirm those of Davis in showing that rabbit virulent strains of pneumococcus Type III can readily gain entrance into the joints. The frequency of such localization increases with the lapse of time following intravenous injection but cannot be correlated directly with the number of cocci in the blood stream. Investigators working with other bacteria have rarely observed arthritis before the 3rd day. In most cases, however, gross clinical manifestations of joint disability were taken as criteria of arthritis. In the present study where direct bacteriological examination was employed, it was found that within 24 hours after infection by the intravenous route nearly all animals have involvement of one or more joints. Cole (13), employing streptococci from a variety of sources, likewise noted that these organisms tended to invade the joints of rabbits early in the disease. In such instances the organisms were demonstrable by culture or smear before there was any gross evidence of articular injury. The experiments of Cole with streptococci and the present ones with pneumococci demonstrate that organisms can be cultured from the joints even though the blood and other organs are sterile.

Our results suggest that pneumococci gain entrance into the joints by direct invasion from the synovial blood vessels. In favor of this interpretation was the presence of organisms in joints before microscopic evidence of inflammation of the synovial membrane or subsynovial tissues was demonstrable. Furthermore, when inflammatory changes were present, they were widespread and did not suggest in any instance a spread from a localized lesion in the subsynovial tissues. This interpretation of the mode of entrance of pneumococci into joints is in accord with the results of other workers (14–16) concerning the entrance of non-viable, particulate, and colloidal materials into joints. The possible direct passage of organisms into joints is greatly enhanced in comparison with other body cavities because of the small amount of tissue intervening between the interior of the joint and the rich subsynovial blood supply, and also because of the

lack of a highly differentiated tissue membrane. These anatomical relationships seemingly afford an explanation for the readiness with which many workers have produced arthritis by the intravenous injection of various organisms into animals. Such "selective localization" has often been interpreted as signifying that the particular organism employed, because of inherent characteristics, was an arthrotropic strain, and therefore must have been of etiological significance in certain forms of arthritis in man. Many past claims for the demonstration of an etiological agent of rheumatoid arthritis and rheumatic fever have been based on such erroneous reasoning.

The greater susceptibility of the knee joints compared with other body cavities to invasion was well demonstrated in the present experiments. Pneumococci were never cultured from the spinal fluids, aqueous humors, or urines of any of the joint-infected animals within 24 hours following inoculation. The pronounced infection of these same body fluids in one animal, 48 hours after infection, however, demonstrates that the anatomical barriers which contain them are not impassable.

The results of the experiments with antiserum indicate that the administration of a single intravenous or intraperitoneal dose of type-specific horse immune serum is ineffective in sterilizing the joints even though some serum constituents do enter the joint cavity (17). The value of antiserum would seem to lie in its ability to abolish the bacteremia and thus reemphasizes the importance of early serum therapy if complications such as pneumococcal arthritis are to be prevented.

SUMMARY

1. Within 24 hours following intravenous inoculation with rabbit virulent strains of pneumococcus Type III, most rabbits develop infections of one or both knees. The frequency of bilateral knee joint involvement increases as the duration of the disease is prolonged.

2. The spinal fluid, aqueous humor, and bladder urine remain sterile at a time when the knee joints contain pneumococci. Subsequently, however, they may be invaded.

3. The administration of a single dose of type specific horse immune serum, at a period when in all probability one or both knee joints contain organisms, appears to be ineffective in bringing about resolution of the infectious process in these sites, even though horse serum constituents may be demonstrated serologically to be present within the joint cavities.

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