

## REACTION OF YOUNG AND ADULT RABBITS TO PNEUMOCOCCI INJECTED INTO THE SKIN

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During recent years chance observations and experimental studies have established the fact that no inflammation or only a comparatively slight one results when irritants are introduced into the skin of very young animals.

Coca, Russell and Baughman (1) noticed that guinea pigs weighing less than 400 gm. react slightly and irregularly to the injection of diphtheria toxin into the skin. Freund (2) and Valtis (3) demonstrated that tuberculous guinea pigs less than 1 month old, do not react at all or only very slightly to tuberculin in the skin test although tuberculous guinea pigs of this age are about as sensitive to the toxic action of old tuberculin injected into the peritoneal cavity as adult tuberculous guinea pigs. Young rabbits do not react with the Arthus reaction to egg white or horse serum (4). Friedberger and Heim (5) made interesting observations on new-born infants and rabbits. They found that no inflammation or but a very slight one results when such powerful irritants as eel serum or mustard oil are injected into the skin of new-born infants or rabbits 1 or 2 days old. The extensive experiments of Adelsberger (6) showed very clearly that young infants react very slightly to turpentine and other irritants when applied to the skin.

The slight reactivity of the skin of infants is significant both from immunological and epidemiological points of view. This is demonstrated by the following observations. Cook, Keith and Ermatinger (7) found that the skin of infants may not react to the injection of a relatively large amount of scarlet fever toxin in spite of the absence of scarlet fever antitoxin in the blood. Von Groer and Kassowitz (8) and Tschertkow and Belgowskaja (9) have shown that in very young infants the Schick test is negative and according to Tschertkow and Belgowskaja this skin test is negative even in the absence of diphtheria antitoxin in the blood. Tschertkow (10) has also observed that infants do not react with inflammation when an autolysate of typhoid vaccine is injected into the skin although such an injection usually produces inflammation in adults. Selter (11) has reported recently that young infants are not sensitive to intracutaneous injections of extracts of various bacteria (typhoid, dysentery, colon, *prodigosus*).

In view of these observations systematic experiments have been undertaken to determine the reaction of very young animals to infection.

## EXPERIMENTAL

To study the infection of the skin in young and adult animals pneumococcus was chosen for it had been shown by Zinsser (12),

TABLE I  
*Effect of Pneumococci Injected into the Skin of Adult and Young Rabbits*

	No. of rabbit	Weight	Amount of culture injected	Local inflammation	Pneumococci in blood smears	Fate of animal
		<i>gm.</i>	<i>cc.</i>			
Adult rabbits	1	2200	0.001	Typical with necrosis	Not found	Survived
	2	2500	0.001	Typical with necrosis	Found	Died in 6 days
	3	2600	0.001	Typical without necrosis	Not found	Survived
	4	3500	0.001	Typical with necrosis	Not found*	Died in 4 days
Rabbits 17 days old	1	140	0.001	None	Found	Died in 1 day
	2	150	0.001	Slight redness, no swelling	Found	Died in 2 days
	3	170	0.001	Slight redness, no swelling	Found	Died in 2 days
	4	175	0.001	Slight redness, no swelling	Found	Died in 2 days
	5	175	0.001	Slight redness, no swelling	Found	Died in 2 days

\* Gelatinous pneumonia; pneumococci not found in the lungs.

Goodner (13) and Klinck (14), that a small amount of a culture of virulent pneumococcus injected into the skin of (adult) rabbits produces a very intense inflammation which, if certain dosage is used, heals as a rule in about 10 days. The typical inflammation was described in detail by Goodner.

In the experiments to be described observations were made on 39 young and 22 adult rabbits.

The age of the young rabbits ranged from 1 to 26 days. They were kept with the mothers. The age of the adult animals was not known. They weighed from

2000 to 3500 gm. and can be considered as fully grown rabbits. Most of the rabbits were of common laboratory stock.

The pneumococcus culture was a freshly isolated strain of Type I; it was grown on rabbit blood bouillon. The amount injected varied from 0.000016 to 0.005 cc. of the culture diluted to the volume of 0.1 cc. Previous to the injection the hair over the skin of the whole abdomen was either shaved or cut with scissors. The

TABLE II  
*Effect of Pneumococci Injected into the Skin of Adult and Young Rabbits*

	No. of rabbit	Weight		Amount of culture injected	Local inflammation	Pneumococci in blood smears	Fate of animal
		gm.	cc.				
Adult rabbits	1	2600	0.0005	Typical without necrosis	Not found	Survived	
	2	2600	0.0005	Typical with necrosis	Not found	Survived	
	3	3400	0.0005	Typical without necrosis	Not found	Survived	
	4	3600	0.0005	Typical without necrosis	Not found*	Died in 15 days	
Rabbits 7 days old	1	70	0.005	None	Found	Died in 1 day	
	2	70	0.005	None	Found	Died in 1 day	
	3	75	0.005	None	Found	Died in 2 days	
	4	80	0.005	None	Found	Died in 1 day	
	5	80	0.005	None	Found	Died in 1 day	
	6	80	0.005	None	Found	Died in 1 day	
	7	80	0.005	None	Found	Died in 1 day	
	8	85	0.005	None	Found	Died in 1 day	
	9	85	0.005	None	Found	Died in 1 day	
	10	90	0.005	None	Found	Died in 1 day	
	11	100	0.005	None	Found	Died in 1 day	

\* Pneumonia; pneumococci not found in the lungs.

animals were observed for 2 weeks, blood smears were examined for pneumococcus daily (with a few exceptions). The results are tabulated in Tables I to VI.

In each experiment, represented in the tables, one group of young rabbits and one group of adults were injected with the same suspension. In the first experiment 0.001 cc. of the culture was injected in both groups. All four adult rabbits had typical local inflammation; one rabbit died in 6 days with bacteriemia, one other rabbit died in 4 days from pneumonia without bacteriemia. The death of the latter rabbit was caused probably not by the injection of pneumococci. Both of these rabbits had typical local inflammation. None of the young rabbits 17 days

old, reacted locally in the way that is characteristic for adult rabbits. The skin was very slightly red only, and no swelling was noticed. Of the young rabbits one died in 1 day and the other four rabbits in 2 days after the infection. The blood of all these contained a large number of extracellular and relatively few intracellular pneumococci (Table I).

In the second experiment four adult and eleven young rabbits, 7 days old, were infected with 0.005 cc. of the culture. All adult rabbits had typical local inflammation; one of them died on the 15th day after infection from pneumonia. None

TABLE III  
*Effect of Pneumococci Injected into the Skin of Adult and Young Rabbits*

	No. of rabbit	Weight	Amount of culture injected	Local inflammation	Pneumococci in blood smears	Fate of animal
		gm.	cc.			
Adult rabbits	1	2600	0.005	Typical without necrosis	Found	Died in 3 days
	2	2900	0.005	Typical without necrosis	Not found	Survived
	3	2900	0.005	Typical with necrosis	Not found	Survived
	4	3100	0.005	Typical with necrosis	Not found	Survived
Rabbits 23 days old	1	200	0.005	None	Found	Died in 2 days
	2	210	0.005	Slight redness, no swelling	Found	Died in 2 days
	3	210	0.005	Slight redness, no swelling	Found	Died in 3 days
	4	240	0.005	Slight redness, no swelling	Found	Died in 3 days
	5	245	0.005	Slight redness, swelling 2 mm. thick	Found	Died in 3 days
	6	255	0.005	None	Found	Died in 3 days
	7	255	0.005	None	Found	Died in 3 days
	8	258	0.005	Slight redness, no swelling	Found	Died in 3 days

of the blood smears of these rabbits showed pneumococci. Of the eleven young rabbits, ten died on the day following the injection and one died 2 days after the injection. All of the young rabbits had pneumococci in their blood. Local inflammation was not noticed (Table II).

In the third experiment four adult and eight young rabbits were infected. The young rabbits were 23 days old. Both groups were infected with 0.005 cc. of the culture. One of the adult rabbits (No. 1) died 3 days after the infection. In the blood smears taken on the 1st, 2nd and 3rd day after the infection there were

many pneumococci both intra- and extracellular. It should be emphasized that the local inflammation was typical. So that in this rabbit the changes that led to death did not modify the development of the local inflammation. Of the young rabbits two died in 2 days and six died in 3 days. The blood smears of all the rabbits contained pneumococci, mostly extracellular. Three of these rabbits showed no local reactions at all; the skin of four rabbits was slightly red without being swollen, and the skin of one young rabbit was red and very slightly thickened (Table III).

In the fourth experiment four adult rabbits were infected with 0.001 cc. and the five young rabbits with 0.000016 cc. of the culture. All the adult rabbits had typical local inflammation and survived the infection without septicemia. None

TABLE IV  
*Effect of Pneumococci Injected into the Skin of Adult and Young Rabbits*

	No. of rabbit	Weight	Amount of culture injected	Local inflammation	Pneumococci in blood smears	Fate of animal
		gm.	cc.			
Adult rabbits	1	2200	0.001	Typical with necrosis	Not found	Survived
	2	2900	0.001	Typical with necrosis	Not found	Survived
	3	2900	0.001	Typical without necrosis	Not found	Survived
	4	3500	0.001	Typical with necrosis	Not found	Survived
Rabbits 8 days old	1	70	0.000016	None	Found	Died in 3 days
	2	100	0.000016	None	Found	Died in 4 days
	3	110	0.000016	None	Found	Died in 4 days
	4	110	0.000016	None	Found	Died in 4 days
	5	110	0.000016	None	Found	Died in 5 days

of the young rabbits showed any local inflammation. All died from 3 to 5 days after the infection with septicemia (Table IV).

In the fifth experiment six adult and ten young rabbits were injected. The young rabbits were 26 days old. In this experiment the amount of culture injected was varied in both groups of rabbits.

Two adult rabbits were infected with 0.005 cc., two with 0.0005 cc. and two with 0.00025 cc. of the culture. All these rabbits developed typical local inflammation; pneumococcus was found in the blood of only one, namely in the rabbit that died 3 days after the infection. Of the young rabbits six were infected with 0.0005 cc. and four with 0.00012 cc. of the culture. In some of these rabbits there was found only slight redness and no swelling at all, and in four rabbits there was a definite edema noticed. Rabbit 4 showed the most conspicuous reaction: slight redness and swelling over an area 30 x 20 mm., elevated about 6 mm. All of these rabbits died with septicemia from 3 to 6 days after infection (Table V).

*Summary of the Five Experiments*

*Adult Rabbits.*—Six rabbits were injected intracutaneously with 0.005 cc., eight with 0.001 cc., six with 0.0005 and two with 0.00025 cc.

TABLE V  
*Effect of Pneumococci Injected into the Skin of Adult and Young Rabbits*

	No. of rabbit	Weight		Local inflammation	Pneumococci in blood smears	Fate of animal
		gm.	cc.			
Adult rabbits	1	2200	0.005	Typical with necrosis	Not found	Survived
	2	2500	0.005	Typical with necrosis	Not found	Survived
	3	2600	0.0005	Typical with necrosis	Not found	Survived
	4	2900	0.0005	Typical without necrosis	Found	Died in 3 days
	5	2950	0.00025	Typical without necrosis	Not found	Survived
	6	3000	0.00025	Typical without necrosis	Not found	Survived
Rabbits 26 days old	1	300	0.0005	Slight redness, no swelling	Found	Died in 3 days
	2	310	0.0005	Slight redness, no swelling	Found	Died in 3 days
	3	315	0.0005	Slight redness, no swelling	Found	Died in 3 days
	4	315	0.0005	Slight redness, swelling 30 x 20 mm. raised 6 mm.	Found	Died in 3 days
	5	325	0.0005	Redness, no swelling	Not found	Died in 6 days
	6	325	0.0005	Slight redness, no swelling	Found	Died in 7 days
	7	330	0.00012	Redness, no swelling	Found	Died in 3 days
	8	330	0.00012	Redness, swelling 12 x 12 mm., raised 3 mm.	Found	Died in 3 days
	9	340	0.00012	Redness, swelling 15 x 15 mm., raised 2 mm.	Found	Died in 3 days
	10	340	0.00012	Redness, swelling 40 x 40 mm., raised 2 mm.	Found	Died in 6 days

of a pneumococcus culture. All of them developed a very extensive local inflammation; three of the rabbits died with septicemia and two without septicemia.

*Young Rabbits, from 1 to 26 Days Old.*—Nineteen young rabbits were injected intracutaneously with 0.005 cc., five with 0.001 cc., six with 0.0005 cc., four with 0.00012 cc. and five with 0.000016 cc. of a pneumococcus culture. None of these rabbits developed the typical local inflammation. Redness with slight swelling was found only in five rabbits, redness without swelling was noticed in fourteen and no

TABLE VI  
*Effect of Pneumococci Injected into the Skin of Adult and Young Rabbits*  
*Adult Rabbits*

No. of rabbits	Dose	Local inflammation	Survived	Died with septicemia	Died without septicemia
	cc.				
6	0.005	Typical	5	1	0
8	0.001	Typical	6	1	1
6	0.0005	Typical	4	1	1
2	0.00025	Typical	2	0	0

*Young Rabbits*

No. of rabbits	Dose	Local inflammation			Survived	Died with septicemia	Died without septicemia
		Absent	Redness, no swelling	Redness and swelling			
	cc.						
19	0.005	14	4	1	0	19	0
5	0.001	1	4	0	0	5	0
6	0.0005	0	5	1	0	5	1*
4	0.00012	0	1	3	0	4	0
5	0.000016	5	0	0	0	5	0

\* 26 days old.

evidence of inflammation was seen in twenty rabbits. All young rabbits died with bacteriemia (Table VI).

DISCUSSION

Goodner and others have demonstrated that the injection of virulent pneumococci into the skin of adult rabbits produces a very extensive inflammation which in some of the animals heals in about 10 days. In others the pneumococci penetrate into the blood stream and cause

death. With the strain and amounts used in the experiments reported in the present paper about one of seven adult rabbits died with septicemia. We have found that young rabbits, from 1 to 26 days old, react very differently to the intracutaneous injection of pneumococci. The inflammation at the site of injection either fails to develop or it is very slight and in all animals without exception the pneumococci penetrate into the blood and kill the rabbits. It was noticed that the pneumococci found in the blood of adult rabbits are mainly in the leucocytes, whereas in the blood of young rabbits they are mainly extracellular.

The observation that young rabbits fail to develop extensive inflammation might be explained by their severe septicemia and by the fact that they live only for a short time after they have been infected. However in those few adult rabbits that died within a few days after infection the septicemia was associated with the usual inflammation at the site of the injection of pneumococci. This observation is in accord with the experiments of Goodner.

In our first experiments an amount of culture was injected both into the young and adult rabbits which was suited to produce extensive inflammation without killing the majority of adult rabbits. In subsequent experiments the amount was varied so that the doses were proportional to the body weights of young and old. Even when this was done, a very small amount of culture, relatively speaking, as little as 0.000016 cc., killed the young rabbits, with septicemia. Since the mortality of young rabbits due to various intercurrent diseases is high (and very frequently the cause of death is not found at autopsy) it is possible that some of the young rabbits would have died during the course of the experiments without having been infected with pneumococci.

The failure of young rabbits to develop extensive inflammation at the site of injection was expected on the basis of observations on the slight reactivity of the skin of young animals. The possible relation of the presence and extent of inflammation to the fate of the animals is of interest. The adult animals develop extensive inflammation and the large majority of them survive the infection without developing a septicemia. The young rabbits do not have inflammation at the site



of the injection, or the inflammation is very slight, and all die with large numbers of pneumococci in the blood stream. It is very probable that the penetration of the bacteria from the skin into the blood stream and the fate of animals is determined by the inflammation at the site of injection of pneumococci. This assumption is supported by experiments which have shown that by means of artificially induced inflammation the spread of bacteria can be checked and animals can be saved from otherwise fatal infection. Opie (15) in a study on the relation of inflammation to immunity found that if, before the injection of streptococci into the peritoneal cavity of rabbits, peritonitis is induced by injection of aleuronat the animals are protected from otherwise fatal bacteriemia.

In addition to the difference in the capacity to develop inflammation young and adult rabbits may differ in regard to the readiness with which non-irritating substances penetrate into the blood from the skin. This may be significant in the reaction of rabbits to pneumococci injected into the skin.

#### CONCLUSIONS

1. Young and adult rabbits react differently to intracutaneous injection of virulent pneumococci. In adult rabbits a very extensive inflammation develops at the site of infection and bacteriemia and death occur only in a relatively few rabbits. Young rabbits fail to develop extensive inflammation and die with bacteriemia.

2. It is probable that the fate of the animals is influenced by the capacity to develop inflammation at the site of injection of pneumococci.

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