FURTHER EXPERIENCES WITH NON-SPECIFIC LOCAL CUTANEOUS IMMUNITY TO STAPHYLOCOCCUS AUREUS

Local Non-Specific Protection

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We have shown previously[1] that plain broth, when used as a skin compress, protected guinea pigs against the effects of subcutaneous injections of lethal doses of *Staphylococcus aureus.* These unspecific compresses protected as efficiently as those made with specific broth filtrates. Whatever production was accomplished either by plain or specific broth filtrates, was localized to the area compressed and lasted at least 24 hours after the removal of the compress. A definite histological difference was shown between the skin of the broth compressed and that of the non-broth compressed animal.

The following experiments were performed (1) to ascertain if the application of other types of local non-specific dressings brought about the same or a modified protection similar to that resulting from broth compresses and (2) to ascertain, if protection did occur, whether the histological picture was comparable to that found in our previous studies with broth-protected animals.

Methods and Materials. Definitions

The animals used were guinea pigs. All were shaved before compresses were applied except where otherwise noted. Because of the possible reaction to the simple act of shaving and irritation, the effects of such preparatory actions were likewise investigated. (Scraping the skin with a scalpel after shaving was considered "irritation.")

[1] Freedlander, S. O., and Toomey, J. A., *J. Exp. Med.*, 1928, **47**, 633.

The amount of bacterial suspension used in each case is noted in the tables. The method of obtaining the suspension has been explained previously.[1] 0.015 cc. of bacteria in 3 cc. of saline was used unless otherwise noted. From time to time, as the stock organism lost its toxicity, a change in the dosage injected became necessary. Whenever long intervals elapsed between experiments, the organism was always found to be less potent and frequently it became necessary to resort to animal passage to enhance its virulence.

The methods of obtaining filtrates, the average weights of the pigs, the type of broth used and its formula were all those described in our previous communication.[1]

Protected pigs were animals which had had broth compresses applied to their abdomens for 48 hours previous to any injection. When mustard plaster was used for protection, strips of prepared commercial mustard plaster were cut, soaked in water and applied to the shaved abdominal wall for 5 minute intervals four times a day for 2 days (8 a.m., 12 p.m., 4 p.m. and 10 p.m.). When animals were treated with saline compresses, the gauze applied was soaked in saline and kept moist for 2 days. Compressing with meat broth extracts, peptone 1 per cent or 10 per cent (respectively) consisted in the local application for 48 hours of gauze soaked with these substances.

Specific filtrate was obtained in the manner described in our previous experiments. In some experiments, bacteria were added to the specific filtrate instead of to saline and the resultant suspension injected subcutaneously into non-protected pigs. Comparative experiments were carried on at the same time with pigs which had been previously protected by broth compresses.

The Lesion

Originally,[1] we described the lesions in terms of one, two and three pluses, representing the end results of the experimentation. In reviewing and again studying the data of these experiments, it became clear that we might adopt a different and more obvious standard of reaction, devoid of any element of the personal equation. Most guinea pig deaths occurred during the first 24 or 48 hours after the bacterial injection and practically all deaths occurred amongst those animals which showed a diffuse swelling of the entire abdominal wall on the 1st day. It was rare that pigs which had a localized inflammatory reaction died even though an abscess might form which later would slough, leaving what we originally described as a three plus end result. Sloughing did not necessarily have any effect on the life of the pig, but inasmuch as it occurred in practically all pigs with a diffuse swelling, the majority of which had a wide-spread ulceration or died, we had previously used its occurrence as a method of meas-

urement. This measurement of reaction ignored other available differences.

After injections into the abdomen, there were obvious early and very striking local differences between the protected and non-protected pigs. This local difference in early reaction was most important as far as the pig's life or protection was concerned. We have redefined and reclassified the lesions as follows, on the basis of these early reactions after injection:

1. Diffuse Reaction.—With this type of reaction, the abdominal skin of the injected pig became raised as a diffuse, soggy, almost cystic swelling within the first 24 hours after injection. There was an absence of the cardinal signs of inflammation other than swelling. There was no clear-cut line of demarcation of the normal from the abnormally involved skin. In appearance, the animals were sluggish, the hair stood out and they would neither eat nor drink.

2. Localized Reaction.—After local injections, such pigs as showed this reaction had a localized swelling with some or all the other cardinal signs of inflammation, *i.e.*, redness, induration, etc. This inflammatory area was from 4 to 8 cm. in diameter, usually the lesion was sharply defined from normal tissue by an indurated border (probably a pyogenic membrane). The animals had local discomfort, but were active and ate well.

Either the diffuse or the localized lesion might go on to abscess formation. The diffuse lesion usually ulcerated and sloughed early, the localized abscesses late.

3. Negative Reaction.—There was no local reaction in these pigs.

I. Results from Compresses of Various Kinds

1. The act of irritating and shaving the abdominal wall of the pig had no effect either one way or the other on local immunity (Table I).

2. Simple water, dry compresses and saline compresses were but slightly protective (Table II).

3. Mustard plasters as compresses were effective, but the results were not so striking as with broth compresses (Table III).

4. Liebig's meat extract protected slightly better than peptone alone and about as well as broth compresses (Table IV).

5. 10 per cent gave no better protection than 1 per cent peptone (Table V).

6. Normal horse serum was found to be as efficient a protector as broth (Table VI).

7. When injected subcutaneously, plain broth and specific broth were absorbed without a trace in less than 24 hours. When unprotected animals were injected with combinations of specific broth filtrate and bacteria, the animal was not protected by specific filtrate, but showed the same reaction as the unprotected pig injected with ordinary solutions of bacteria and saline (Table VII).

These last experiments are in contrast with the constant protection obtained in the controls by the application of broth for 48 hours previous to injection.

8. Protection from broth compresses lasted at least 120 hours (Table VIII).

9. When animals were injected with saline suspensions of bacteria and an effort was made to protect them with local broth applications after this injection, the mortality rate was extremely high as compared with that in the control pigs (Table IX).

10. Pigs which recovered from injections of staphylococcus suspensions were again injected with the same organisms within 30 days after the clearing of the previous lesion. Such reinjected pigs seemed still to have some protection (Table X).

II. Microscopic Results

Microscopically, the slides from the control animal treated with broth showed the same picture described in our previous article. When any agency protected the animal as well as broth did, the local reaction was the same as that in broth-protected animals. It could be said in general that any protective dressing used, produced a histological picture somewhat roughly proportionate to its ability to stimulate the tissue. Where complete protection occurred as with horse serum, plain broth and Liebig's extract, the same histological picture was produced as with specific broth or non-specific broth.

In the main, simple bandaging, plain water and saline compresses gave rise at best to but a slight reaction, a moderate increase in the clasmatocytes and a barely noticeable thickening of the epidermal coats. Where protection was definite with these mild stimulants, the pathological picture was the same as though broth had been used. Examination of slides taken at various intervals after protection showed

clasmatocytes in great numbers as long as 16 days after protection was discontinued.

COMMENT

Besredka states that specific filtrates are bactericidal *in vivo* and when bacteria and specific broth filtrates are injected locally, there is no response on the part of the tissues. One, as it were, neutralizes the other. That there is no such protection is shown by our experiments. Although plain broth and specific broth filtrate are readily absorbed as such, the addition of viable staphylococcus to either and the injection of the mixture subcutaneously was followed either by death or the occurrence of a diffuse lesion in the test animal.

Imschenetsky[2] who worked with topical applications of Besredka's "staphylococcus broth virus," stated that his conclusions were the same as those of Freedlander and Toomey[1] except that in addition to what these authors described, he noted leucocytes in the epithelial layer, scattered and sometimes accumulated beneath the stratum corneum. He further stated that he, unlike us, was unable to find any fibroblastic proliferation. He described the effect of "staphylococcus broth virus" application, while in our paper, we pictured the effect of broth applications. Our subsequent work showed that there was no difference in skin reaction to plain broth compresses as compared to "specific staphylococcus broth compresses" (Besredka's staphylococcus antivirus), either grossly or microscopically so that Imschenetsky's error of comparison is not of any great moment. That fibroblastic proliferation existed may be noted from Fig. 11, Plate 30 of our original article[1] where such a reaction is photographed.

We also noted an increase in polymorphonuclear cells, for in our first article, we stated that "while there was a moderate number of polymorphonuclears and small mononuclear leucocytes, there was, especially in the subcutis, a marked increase in the number and size of the clasmatocytes and elongated tissue cells." This scattered infiltration of leucocytes was not the predominating reaction and hence was not stressed. We did not perceive the distinctive reaction of the hair follicles noted by the author.

[2] Imschenetsky, A., *Z. ges. exp. Med.*, 1929, **69**, 113.

Imschenetsky found that the longer applications were applied, the greater were the skin changes, a dictum to which we can subscribe. That compressing with such simple materials as normal sodium chloride solution may sometimes give reactions comparable to those obtained by broth is also shown by our experiments, but these are the exceptions rather than the rule and where such protection occurs, we would be inclined to attribute it to the pressure applied, rather than to the heat of the compress as claimed by the author.

The fact that we have obtained slight or great immunity by various procedures and materials would properly explain conflicting good results obtained with divers substances.

Guinea pigs which are "broth-protected" prior to bacterial injection live, but animals broth-protected after injection die. This parallels Gay's experience in experimental pleural infections with streptococcus.

That some materials excite the tissues to relatively great reaction is obvious from these and our previous experiments.

CONCLUSIONS

1. Many substances besides the specific broth filtrates of Besredka can be utilized as topical applications to protect guinea pigs from the effects of massive doses of staphylococcus given subcutaneously. (Plain broth, peptone 10 per cent, peptone 1 per cent, Liebig's meat extract, mustard plaster and normal horse serum.)

2. Where such protection occurs, no matter what the stimulus is, the local skin reaction microscopically is the same as that previously described for broth compresses.

3. Many topical applications of such substances as saline, water, plain compresses, etc., may confer slight protection on an animal.

4. Specific filtrates (Besredka) confer no protection on the experimental animal if applied at the time of inoculation or thereafter.

5. The local protection described in our experiments is non-specific in its nature.

TABLE I

Protective Effect of Various Simple Procedures

Procedure	Dosage	No. of exp.	Date injected	No. pigs used	Reaction			
					Diffuse	Local	Neg.	Died
Shaved, not irritated, broth-protected	2 cc. broth culture suspension, not standardized	14	10/28/24	5	0	5	0	0
	Same as above	15	11/ 1/24	5	0	5	0	0
Total.....				10	0	10	0	0
Shaved, not irritated, not protected	For dosages, see corresponding experiments	14	10/28/24	5	5	0	0	1
		15	11/ 1/24	5	5	0	0	0
Total.....				10	10	0	0	1
Shaved, irritated, not protected	For dosages, see corresponding experiments	14	10/28/24	5	4	1	0	0
		15	11/ 1/24	5	4	1	0	0
Total.....				10	8	2	0	
Not shaved, not irritated, not protected	For dosage, see corresponding experiment	14	10/28/24	5	5	0	0	1

TABLE II

Effect of Dry Compresses, Saline Compresses and Plain Water Compresses

Procedure	Dosage	No. of exp.	Date injected	No. pigs used	Reaction			
					Diffuse	Local	Neg.	Died
Protected by saline compresses	2 cc. of broth suspension	15	11/ 1/24	5	1	4	0	0
	Same as above	16	12/24/24	5	4	1	0	1
	" " "	17	1/12/25	5	5	0	0	0
	0.03 cc. bacteria in 3 cc. broth	64a	2/ 5/27	4	2	2	0	0
	Same as above	83	1/19/28	10	6	4	0	3
	" " "	84	2/ 4/28	10	7	3	0	4
Total.....				39	25	14	0	8
Protected by plain water compresses	For dosages, see corresponding experiments	15	11/ 1/24	5	5	0	0	0
		16	12/24/24	5	5	0	0	0
		17	1/12/25	5	5	0	0	0
	3 cc. of saline suspension	19	3/14/26	10	4	6	0	0
Total.....				25	19	6	0	0
Protected by dry compresses	For dosages, see corresponding experiments	17	1/12/25	5	5	0	0	0
		19	3/14/26	10	4	6	0	0
		64a	2/ 5/27	4	3	1	0	1
Total.....				19	12	7	0	1
Controls: protected by broth compresses	For dosages, see corresponding experiments	15	11/ 1/24	5	0	5	0	0
		16	12/24/24	5	0	5	0	0
		17	1/12/25	5	1	4	0	1
		64a	2/ 5/27	4	0	4	0	0
		83	1/19/28	10	1	9	0	2
		84	2/ 4/28	10	0	8	2	1
Total.....				39	2	35	2	4
Controls: not protected	For dosages, see corresponding experiments	15	11/ 1/24	5	5	0	0	0
		16	12/24/24	5	5	0	0	0
		17	1/12/25	5	5	0	0	0
		19	3/14/26	10	10	0	0	1
		64a	2/ 5/27	4	4	0	0	0
		83	1/19/28	10	8	2	0	6
		84	2/ 4/28	10	9	1	0	7
Total.....				49	46	3	0	14

TABLE III

Protective Effect of Mustard Plaster Compresses

Procedure	Dosage	No. of exp.	Date injected	No. pigs used	Reaction			
					Diffuse	Local	Neg.	Died
Mustard plaster compresses	0.015 cc. bacteria in 3 cc. saline	69	2/17/27	8	6	2	0	2
	Same as above	71	3/31/27	8	2	6	0	1
Total.....				16	8	8	0	3
Protected by broth compresses	For dosages, see corresponding experiments	69	2/17/27	8	1	7	0	0
		71	3/31/27	8	1	7	0	1
Total.....				16	2	14	0	1
Controls: no protection	For dosages, see corresponding experiments	69	2/17/27	8	8	0	0	4
		71	3/31/27	8	8	0	0	1
Total.....				16	16	0	0	5

TABLE IV

Protective Effect of Peptone Compresses

Procedure	Dosage	No. of exp.	Date injected	No. pigs used	Reaction			
					Diffuse	Local	Neg.	Died
Protected by 1 per cent peptone compresses	0.015 cc. bacteria in 3 cc. saline	85	2/29/28	10	5	5	0	2
Protected by Liebig's meat extract, 1 per cent	Same as above	85	2/29/28	10	1	3	6	0
Controls: broth-protected	" " "	85	2/29/28	10	0	4	6	0
Controls: not protected	" " "	85	2/29/28	10	10	0	0	4

TABLE V

Comparative Protective Effects of Peptone Dilutions, 1 and 10 Per Cent

Procedure	Dosage	No. of exp.	Date injected	No. pigs used	Reaction			
					Diffuse	Local	Neg.	Died
Protected by 1 per cent peptone compresses	0.015 cc. bacteria in 3 cc. saline	87	3/27/28	10	3	7	0	3
Protected by 10 per cent peptone compresses	Same as above	87	3/27/28	10	3	7	0	4
Protected by broth compresses	" " "	87	3/27/28	10	0	9	1	0
Controls: not protected	" " "	87	3/27/28	10	10	0	0	4

TABLE VI

Illustrating the Protective Power of Normal Horse Serum

Procedure	Dosage	No. of exp.	Date injected	No. pigs used	Reaction			
					Diffuse	Local	Neg.	Died
Protected with normal horse serum compresses	0.015 cc. bacteria in 3 cc. saline	120	4/24/29	8	1	7	0	0
	Same as above	121	10/ 8/29	5	0	5	0	0
Total.....				13	1	12	0	0
Controls: unprotected	For dosages, see corresponding experiments	120	4/24/29	8	7	1	0	3
		121	10/ 8/29	5	3	2	0	0
Total.....				13	10	3	0	3

TABLE VII

Illustrating the Neutralizing Effect of Specific Broth Filtrates (Besredka)

Procedure	Dosage	No. of exp.	Date injected	No. pigs used	Reaction			
					Diffuse	Local	Neg.	Died
Group A: protected by broth compresses; positive controls	0.015 cc. bacteria in 3 cc. saline	77	7/11/27	10	1	5	4	0
Group B: controls; not protected	Same as above	77	7/11/27	10	6	2	2	1
Group C: not protected	0.015 cc. bacteria in 3 cc. specific broth	77	7/11/27	10	10	0	0	4
Group D: not protected	0.015 cc. bacteria in 3 cc. plain broth	77	7/11/27	10	10	0	0	5
Group E: injected to time absorption	3 cc. plain broth	77	7/11/27	10	0	0	10	0
Group F: injected to time absorption	3 cc. specific broth	77	7/11/27	10	0	0	10	0

TABLE VIII

Illustrating the Length of Time Protection Lasts after Broth Compresses

Procedure	Dosage	No. of exp.	Date injected	No. pigs used	Reaction			
					Diffuse	Local	Neg.	Died
Broth compresses, protected pigs, injected 24 hrs. after protection ceased	0.015 cc. bacteria in 3 cc. saline	76	6/ 7/28	6	0	2	4	0
Controls: not protected				6	6	0	0	2
72 hrs. after protection ceased	Same as above	76	6/7 /28	3	0	2	1	0
Controls: not protected				3	3	0	0	1
120 hours after broth-protection ceased	" " "	76	6/ 7/28	3	0	3	0	0
Controls: not protected				3	3	0	0	1
168 hours after broth-protection ceased	" " "	76	6/ 7/28	3	1	2	0	1
Controls: not protected				3	3	0	0	0
216 hours after broth-protection ceased	" " "	76	6/ 7/28	2	2	0	0	2
Controls: not protected				2	2	0	0	2

TABLE IX

Effect of Broth Protection Applied after Bacterial Injection

Procedure	Dosage	No. of exp.	Date injected	No. pigs used	Reaction			
					Diffuse	Local	Neg.	Died
Controls: protected by broth compresses before injection	0.015 cc. bacteria in 3 cc. saline	74	5/ 5/27	8	0	8	0	2
	Same as above	75	5/24/27	8	1	7	0	0
	" " "	89	5/21/28	12	0	7	5	0
Total.....				28	1	22	5	2
Controls: not protected *before* injection	For dosages, see corresponding experiments	74	5/ 5/27	8	8	0	0	8
		75	5/24/27	8	8	0	0	1
		89	5/21/28	12	7	5	0	0
Total.....				28	23	5	0	9
Animals not protected *before*, but *after* injection by applying broth compresses to the injected area	For dosages, see corresponding experiments	74	5/ 5/27	8	8	0	0	8
		75	5/24/27	8	5	0	3	6
		89	5/21/28	16	11	5	0	14
Total.....				32	24	5	3	28

TABLE X

Reinjection Experiments to Illustrate the Length of Time Protection Lasts

Procedure	Dosage	No. of exp	Date injected	No. pigs used	Reaction			
					Diffuse	Local	Neg.	Died
These animals had been previously injected and had recovered. Injected 30 days previous	0.015 cc. bacteria in 3 cc. saline	88	5/ 4/28	10	5	5	0	1
Injection 21–40 days previous	Same as above	33	9/ 5/28	9	0	9	0	0
Controls: not protected	" " "	88	5/ 4/28	10	10	0	0	6