THE EFFECT OF TESTICULAR EXTRACT ON EXPERIMENTAL TUBERCULOSIS IN RABBITS. I. SKIN LESIONS *

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Duran-Reynals [1, 2, 3] has shown that there exists in the testes of normal rabbits a substance that has the remarkable power of enhancing skin infections produced by vaccine virus and by staphylococci. Hoffman [4] confirmed the observation on the enhancement of vaccine virus and extended the work to show that this phenomenon is equally effective with the viruses of vesicular stomatitis, Borna disease, and herpes; and in the case of the viruses of herpes, Borna disease, and vaccinia, the enhancement applied also to central nervous system infections. Pijoan [5] reported that this substance, when added to cultures of twenty different bacteria, caused enhancement to a high degree of the resulting intradermal lesions. Hoffman and Duran-Reynals [6] found that the Berkefeld filtrate of testicular extract was equally as effective as the unfiltered emulsion. To this enhancing substance Ledingham and Barratt [7] have given the name "Reynals factor."

Since all the bacteria used in the above mentioned experiments were those that usually produce a more or less uniform, acute reaction, it was felt that it might be of interest to apply this enhancing substance to an organism giving an entirely different type of reaction, *i.e.*, to the tubercle bacillus. Consequently experiments were designed to determine what effect, if any, testicular extract might have upon the character of the lesions resulting from the intradermal injection of tubercle bacilli in rabbits.

METHODS AND MATERIALS

The tubercle bacilli used in these experiments consisted of avian, human, and bovine strains. The organisms were grown on a modified egg medium † and were from 1 to 2 months old at the time of

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† The modified egg medium is prepared as follows:

Potatoes	250 gm.
Distilled water	500 cc.
Glycerin	20 cc.

Autoclave 30 minutes under 15 lb. pressure.

To 50 cc. of the above potato glycerin extract add 50 cc. bouillon and 5 eggs. Put in tubes and inspissate. Add 1 cc. distilled water to each tube.

Autoclave 20 minutes under 15 lb. pressure.

use. A heavy, fresh saline emulsion of the organisms was used for inoculation.

Testicular extract was freshly prepared from testes of healthy adult rabbits as follows. The testes were removed aseptically,* and after the fat was dissected away they were weighed, minced and ground with sand in a sterile mortar. Sufficient Locke's solution was added to make a 1:10 suspension and the mass was then centrifuged at high speed for 15 minutes. The supernatant fluid was passed through a Berkefeld Y filter. This filtrate constituted the extract used in the experiments and it was tested for sterility before use.

Injection masses for each of the avian, human, and bovine strains were prepared for inoculation as follows. The saline suspension of the organisms was divided into two equal parts. To the first part was added an equal volume of testicular extract, and to the second, an equal volume of sterile normal saline. Thus the first mass served as the experimental material and the second as its control. For each strain of organism the injection masses contained approximately the same number of organisms and differed from each other only in that the experimental mass contained testicular extract, whereas the control did not.

Nine healthy, young adult rabbits were used in the experiments. The flanks were clipped and then shaved. The animals were divided into three groups of three animals to each group. One group was used for the avian, one for the human, and one for the bovine strains.

For the experiment in which the avian strain was employed 1 cc. of the experimental injection mass containing a saline suspension of organisms plus an equal volume of testicular extract was injected intradermally in one area, and 1 cc. of the corresponding control was injected in like manner in another part of the shaved skin on the same side. Two animals were thus inoculated. The third received similar injections, with the exception that both sides of the animal were inoculated.

The three animals receiving the bovine strain were inoculated in similar fashion with two control and two experimental injections on either side.

The remaining three rabbits received four inoculations consisting of 1 cc. of the testicular extract-bacilli suspension (human strain)

* All operations on animals were carried out under full ether anesthesia.

on each side, and likewise four inoculations of saline-bacilli emulsion. Thus each animal received a total of sixteen inoculations. The animals were kept in cages and fed the usual laboratory diet.

Twelve to eighteen days were allowed for the disease to develop, during which time the size and character of the lesions were noted from day to day. The animals were then killed and tissue from both the experimental and the control lesions was removed and fixed in a mixture of alcohol and formalin (9 parts alcohol and 1 part formalin). Celloidin sections were cut and stained by the carbol fuchsin method of Ziehl-Neelson.

It was noted at the time of the inoculations that all injections containing testicular extract diffused so rapidly that no wheal was visible at the end of 2 minutes, whereas those injections without testicular extract resulted in wheals that were visible as long as 30 minutes after inoculation.

Results

The lesions resulting from the injection of organisms with testicular extract were without exception more severe and much more extensive than the respective control lesions. This difference was found to be constant, irrespective of the type of organism used. All early lesions in every series were similar in appearance, but as the disease progressed, those resulting from the inoculation of bacilli with testicular extract became more extensive, showed necrosis and ulcerated earlier than the corresponding control lesions.

The lesions resulting from the inoculation of avian or bovine bacilli were more extensive and more severe than those resulting from the injection of the human strain in both the control and experimental injections.

The microscopic changes consisted of a marked infiltration with lymphocytes and monocytes, with occasional tubercle formation. There were also varying degrees of necrosis in some instances. Acid-fast bacilli (occurring singly or in clumps) were present in all lesions. There was no essential qualitative microscopic difference in either the avian, human or bovine lesions and their respective controls.

In order to express the enhancement phenomenon in more concrete terms the average plane area of the lesions in every series was computed. Those due to the avian bacilli plus saline averaged

TABLE I

Avian Strain

Rabbit	Inoculation		Duration	Size and character of lesions	
	Equal parts emulsion plus saline	Equal parts emulsion plus testicular extract		Emulsion plus saline	Emulsion plus testicular extract
	cc.	*cc.*	*days*	*cm.*	*cm.*
A	1	1	12	3 x 3	15 x 4 Marked ulceration
B	1	1	12	4 x 4 Ulcerated	12 x 6 Marked ulceration
C	1	1	13	3 x 2.5	6 x 3.5
C	1	1	13	3 x 2	6 x 3 Ulcerated

TABLE II

Bovine Strain

Rabbit	Inoculation		Duration	Size and character of lesions	
	Equal parts emulsion plus saline	Equal parts emulsion plus testicular extract		Emulsion plus saline	Emulsion plus testicular extract
	cc.	*cc.*	*days*	*cm.*	*cm.*
D	1	1	15	3 x 3 Ulcerated	12 x 6 Marked ulceration
D	1	1	15	3 x 2.5 Ulcerated	8 x 3.5 Ulcerated
E	1	1	16	3 x 1.5	4.5 x 4 Moderate ulceration
E	1	1	16	2.5 x 1.5 Slight ulceration	5 x 3 Moderate ulceration
F	1	1	16	1.5 x 1.5	4.5 x 4 Slight ulceration
F	1	1	16	1.5 x 1	4 x 2.5 Slight ulceration

9.6 sq. cm. in diameter, whereas those following injection of the bacilli-testicular extract mass averaged 42.7 sq. cm. Thus, the latter lesions were 4.3 times the size of their controls. With the bovine

TABLE III

Human Strain

Rabbit	Site for injection	Inoculation				Duration	Size and character of lesions	
		Equal parts emulsion plus saline		Equal parts emulsion plus testicular extract			Emulsion plus saline	Emulsion plus testicular extract
		No.	*Amt. in cc.*	*No.*	*Amt. in cc.*	*days*	*cm.*	*cm.*
G ...	Right side	4	1	4	1	14	Separate nodules about 1.3 x 1.3 Slightly ulcerated	Confluent lesion 9 x 3.5 Moderate ulceration
G ...	Left side	4	1	4	1	14	Separate nodules about 1.2 x 1.2 Slight ulceration	Confluent in denoted lesion 9.5 x 3.2 Moderate ulceration
H ...	Right side	4	1	4	1	14	Separate nodules about 1.5 x 1.5 Slight ulceration in centers	Confluent lesion 9 x 3.5 Marked ulceration
H ...	Left side	4	1	4	1	14	Separate nodules about 1.5 x 1.5 Slight ulceration in centers	Confluent lesion 9 x 4 Marked ulceration meeting in midline
I ...	Right side	4	1	4	1	18	Separate nodules 1.5 x 1 Slight ulceration in centers	Confluent lesion 8.5 x 3.5 Moderate ulceration
I ...	Left side	4	1	4	1	18	Separate nodules 1.5 x 1.5 Slight ulceration in centers	Confluent lesion 9.2 x 3.4 Moderate ulceration

strain the controls averaged 4.75 sq. cm., while those resulting from testicular extract injection were 28.6 sq. cm. The latter were therefore 5.6 times the controls in size. The lesions due to human bacilli were more difficult to measure with any reasonable degree of accu-

racy, because a large number of inoculations were made in a small space and became confluent. However, as accurately as could be determined, the control lesions averaged 1.82 sq. cm. in area, while the experimental lesions averaged 7.875 sq. cm. The size of the latter was 4.3 times that of the controls.

The results of our experiments are represented in tabular form (see Tables I, II and III).

Discussion

Considerable evidence has been presented in the literature in support of the fact that testicular extract will increase the severity and extent of acute lesions resulting from pyogenic bacteria or viruses.[1-5] Our results clearly demonstrate that testicular extract is equally effective in enhancing the lesions resulting from the intradermal injection of avian, bovine or human strains of tubercle bacilli in rabbits. These organisms normally produce lesions that differ from those previously reported in that the incubation period is longer and the reaction is of an entirely different type. Thus, the enhancing effect of testicular extract in infections appears to be general, whether such infections be acute or chronic, bacterial or viral.

It is well known that the avian and bovine strains are much more virulent for rabbits than the human strain. We have found this same relative difference in the severity of the resulting skin infections due to these organisms. Regardless of the size of the control lesions those resulting from the injection of bacilli together with testicular extract were approximately five times as extensive. From these observations and the work of others, it appears conclusive that the extract of normal rabbit testes possesses some property of enhancing the severity of representative lesions from all classes of infections.

It was noted at the time of inoculations that the injection masses containing testicular extract diffused very rapidly, which is in accord with the observations of Hoffman and Duran-Reynals [6] and of McClean.[8] It is possible that the resulting enhancement of the infections may be due either to some action of the active principle of testicular extract on the organisms or on the host or both. It seems impossible to say on the basis of our observations whether the enhancement is due to a wider distribution of organisms, an increase in virulence, a decreased resistance of the host, or a combination of these factors. The reader is referred to papers by Hoffman, Duran-

Reynals, and McClean for a discussion regarding the mechanism of action of testicular extract.

The results of these experiments suggest the possibility of employing this enhancing factor in a clinical connection in animal inoculation of body fluids and exudates where such a procedure is necessary to make a diagnosis of tuberculosis.

Conclusions

1. When tubercle bacilli together with testicular extract are injected into the skin of rabbits marked enhancement of the resulting lesions occurs.

2. This enhancement takes place equally well with the human, bovine, and avian strains.

3. The practical application of this enhancement phenomenon in laboratory diagnosis is worthy of consideration.

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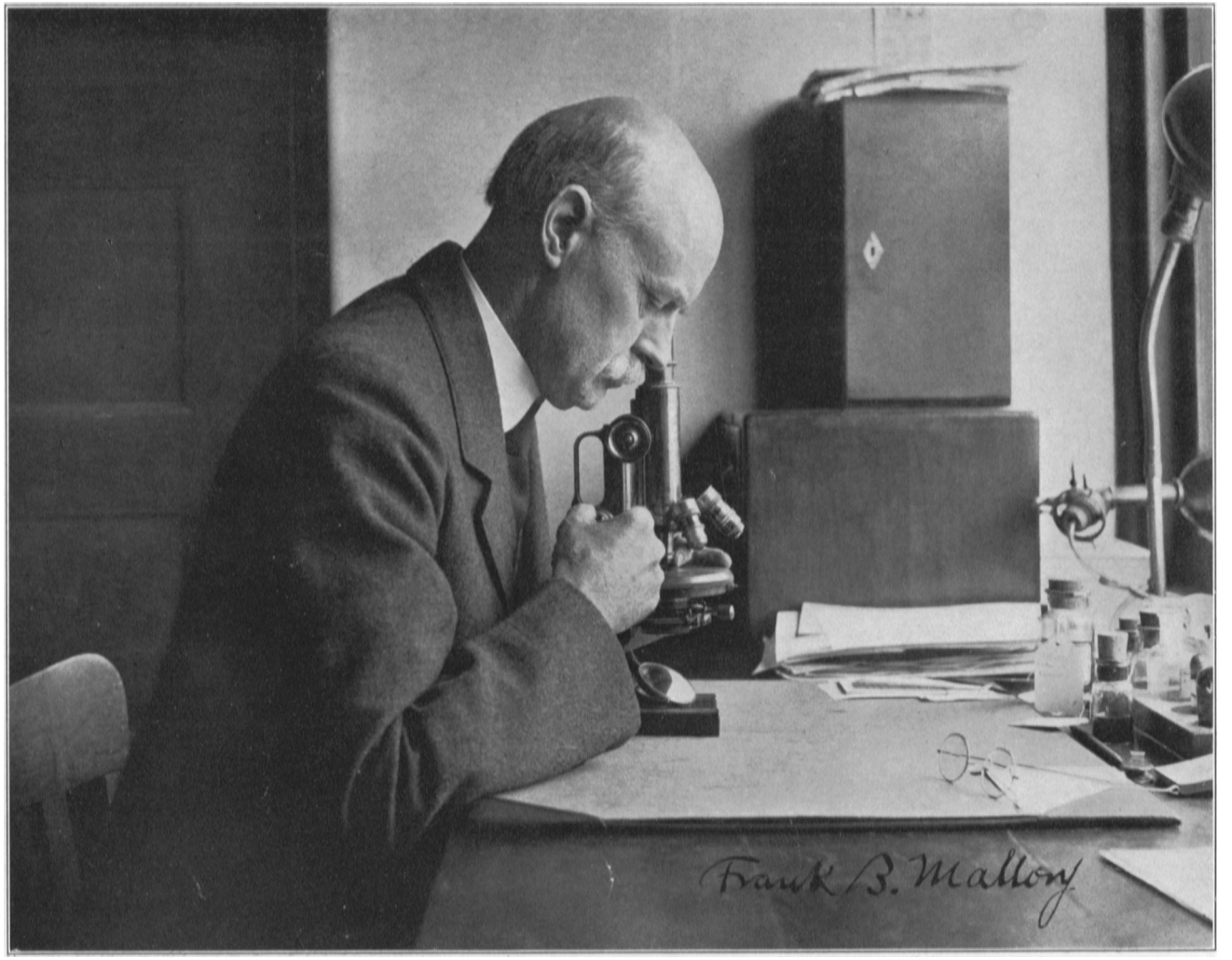

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