

FURTHER STUDIES ON THE INFLUENCE OF TESTICLE  
EXTRACT UPON THE EFFECT OF TOXINS, BACTERIA,  
AND VIRUSES, AND ON THE SHWARTZMAN AND  
ARTHUS PHENOMENA

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PLATES 23 TO 25

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While there is general agreement on the enhancing effect of testicle extract on infective processes (1), the literature contains conflicting reports on the effect of the testicle factor on the lesions produced by toxins and enzymes. In collaboration with Hoffman (2), we recorded the fact that testicle extract did not influence the type of lesions produced by *B. coli* toxin or trypsin injection in the skin of rabbits, nor did it enhance the action of tetanus toxin in guinea pigs. However, McClean (3) has reported that testicle extract definitely enhances the local lesions produced by intracutaneous injection of diphtheria toxin. More recently Bier (4) has claimed that the Shwartzman phenomenon is intensified if the testicle factor be added to the "preparatory" intracutaneous injection of the toxin.

We have attempted to elucidate these apparently conflicting findings by investigating the effect of varying concentrations of the agents employed in the experiments, as this seemed to be the most likely variable.

*Material and Methods*

The essential part of the experimental technique consisted in intracutaneous injection of constant amounts of progressive dilutions of the infective or toxic agents mixed with rat or bull testicle extract prepared in the usual way. The resulting lesions were compared with those produced by the same dilutions of the agents mixed with Ringer's solution or water as controls. This procedure was followed in the experiments with bacteria and viruses and in those with horse serum, in testing the Arthus phenomenon. In other tests the marked individual differences in the response of normal or prepared rabbits to injection of bacterial toxins

or the comparative benignity of the skin reactions to certain foreign sera made dilution of the agents unnecessary. The areas of the lesions were determined at suitable intervals and recorded in square centimeters.

*Effect of Testicle Extract on Lesions Produced by Bacterial Toxins and on the Shwartzman Phenomenon*

If a small amount of certain bacterial toxins is injected intracutaneously and 24 hours later an intravenous injection of the same or another toxin is given, a profound reaction takes place in the injected skin area. This so called Shwartzman phenomenon has been studied both as concerns the influence of testicle extract on the initial lesions from the intracutaneous or preparatory injection, and its influence on the secondary phenomenon.

*Experiment.*—Rabbits were prepared by the intracutaneous injection of 0.25 cc. of filtrate from a 6 day old broth culture of *B. coli* mixed with testicle extract, and control animals received the same amount of toxin diluted with Ringer's solution. After 24 hours the sizes of the lesions were recorded and the animals were then given an intravenous injection of 2 cc. per kilo of body weight of the same toxin. The resulting changes in the skin lesions were recorded after another 8 hours. The results of the two series of observations in the test and control animals are given in Table I.

In order to eliminate the individual variations between animals, each rabbit of the next group received the test inoculation of toxin and testicle extract and also the toxin diluted with Ringer's solution. The results are recorded in Table II.

In analyzing the results it is necessary to separate the effect of testicle extract on lesions produced by the preparatory intracutaneous injections and the action of the same factor on the lesion developing in the same area after the intravenous injection of the bacterial toxin (Shwartzman phenomenon). Nevertheless, the same principle was manifest in both instances. The area of the lesion produced by the toxin with testicle extract and the extent of the Shwartzman phenomenon were undoubtedly larger than in the controls, but what the lesions had gained in extent they had lost in intensity. This was particularly true of the Shwartzman phenomenon, where instead of the well defined and severe edematous and hemorrhagic character, the lesions were flat, diffuse, and mild. In the animals in which the con-

TABLE I  
*Effect of Testicle Extract on the Lesions Produced by B. coli Toxin and on the Shwartzman Phenomenon (First Group)*

Rabbit No.	Preparatory intracutaneous injection			Toxic injection (2 cc. per kilo of body weight)	
	Material injected with 0.25 cc. of bacterial filtrate	Area of lesion after 24 hrs. sq. cm.	Character of the lesions	Area of lesion after 8 hrs. sq. cm.	Character of the lesions
1	0.25 cc. Ringer's solution	6.0	Strongly congestive, clearly defined	10.8	Strongly hemorrhagic and necrotic, clearly defined. Animal sick
2	0.25 " "	8.0	Strongly congestive, clearly defined	17.6	Strongly hemorrhagic, clearly defined. Animal died in 2 hrs.
3	0.25 " "	8.9	Strongly congestive, clearly defined	9.9	Strongly hemorrhagic, clearly defined. Animal sick
4	1.00 " "	4.9	Mildly congestive, clearly defined	6.5	Strongly hemorrhagic, clearly defined
5	1.00 " "	12.6	Mildly congestive, clearly defined	20.4	" " "
6	1.00 " "	17.0	Mildly congestive, clearly defined	?	Negative
7	0.25 " testicle extract	50.0	Erythematous, diffuse, flat	100.0	Strongly hemorrhagic, diffuse, flat. Animal died in 8 hrs.
8	1.00 " "	38.5	Strongly congestive, diffuse, flat	66.5	Mildly hemorrhagic, diffuse, flat. Animal very sick
9	1.00 " "	50.0	Faintly erythematous, diffuse, flat	81.0	Mildly hemorrhagic, diffuse, flat. Animal very sick
10	1.00 " "	36.9	Faintly erythematous, diffuse, flat	16.0	Faintly erythematous, one hemorrhagic spot
11	1.00 " "	30.0	Faintly erythematous, diffuse, flat	15.0	Faintly erythematous, one hemorrhagic spot

TABLE II  
*Effect of Testicle Extract on the Lesions Produced by B. coli Toxin and on the Shwartzman Phenomenon (Second Group)*

Rabbit No.	Preparatory intracutaneous injection			Toxic injection (2 cc. per kilo of body weight)	
	Material injected with 0.25 cc. of bacterial filtrate	Area of lesions after 24 hrs. sq. cm.	Character of the lesions	Area of lesions after 8 hrs. sq. cm.	Character of the lesions
1	0.25 cc. Ringer's solution 0.25 " testicle extract	4.9 ?	Congestive, clearly defined Faintly erythematous, very diffuse	12.6 64.4	Moderately hemorrhagic, clearly defined " " flat, diffuse
2	0.25 " Ringer's solution 0.25 " testicle extract	3.3 ?	Congestive, clearly defined Almost unnoticeable erythema	4.0 9.2	" " clearly defined Faintly erythematous, one small hemorrhagic spot
3	0.25 " Ringer's solution 0.25 " testicle extract	5.3 49.0	Congestive, clearly defined Faintly erythematous, very diffuse	5.6 —	Congestive, clearly defined Practically negative reaction
4	0.25 " Ringer's solution 0.25 " testicle extract	5.7 25.0	Erythematous, clearly defined Erythematous, very diffuse		Animal died 2 hrs. after injection. No reaction

trol lesion was mild, the one resulting from the toxin-testicle extract mixture was extremely benign or in some cases completely suppressed.

In an additional experiment carried out on four rabbits the procedure was varied by injecting the testicle extract into the prepared area at the same time as the intravenous toxin injection was given. This provides a similar spreading out of the Shwartzman reaction with a corresponding lessening of the severity, or even suppression of the reaction.

It may be concluded from these three sets of experiments that testicle extract has no true enhancing property on lesions produced by bacterial toxins used or on the subsequent Shwartzman phenomenon.

#### *Effect of Testicle Extract on the Skin Reactions to Foreign Sera*

Human serum, injected into the skin of a rabbit, produces a well defined lesion which reaches its maximum reaction after 24 hours and regresses after 2 to 3 days. Horse serum induces much the same type of reaction but of a much milder nature. These reactions afforded an opportunity for testing the effect of testicle extract on the intensity of the skin lesions induced by inanimate material.

*Experiment.*—Human and horse sera were diluted with equal parts of testicle extract and with Ringer's solution for control. Each rabbit received an intracutaneous injection of each of the sera with testicle extract and with the serum diluted with Ringer's solution. The results after 48 hours are summarized in Table III.

These tests emphasize the results obtained in the first group of experiments. The spreading or diffusion of the injurious agent brought about by the testicle factor reduces the intensity of the reaction; and if this causes ordinarily but a mild lesion it is spread over so large an area as result of the testicle extract that its effects become barely discernible.

#### *Effect of Testicle Extract on the Arthus Phenomenon*

As a further test of the effect of spreading, experiments have been made on the Arthus phenomenon.

*Experiment.*—Rabbits were sensitized by the injection of 5 cc. of horse serum subcutaneously at 6 day intervals until each had received five injections. 13

TABLE III  
*Effect of Testicle Extract on the Reactions Produced by Intracutaneous Injections of Human and Horse Serum in the Normal Rabbit*

Rabbit No.	Material injected		Area of resulting reactions <i>sq. cm.</i>	Character of the reactions
1	1 cc. horse serum plus 1 cc. Ringer's solution	1 " " 1 " testicle extract	12.9 ?	Congestive, edematous, clearly defined Almost unnoticeable erythema
2	0.5 " " " 0.5 " Ringer's solution	0.5 " " " 0.5 " testicle extract	6.2 0.0	Congestive, edematous, clearly defined Practically no reaction
3	0.2 " " " " 0.2 " Ringer's solution	0.2 " " " " 0.2 " testicle extract	4.0 ?	Slightly congestive and edematous, clearly defined No reaction
4	1 " human " " " 1 " Ringer's solution	1 " " " " 1 " testicle extract	18.0 ?	Strongly congestive and edematous, clearly defined Faintly erythematous
5	0.5 " " " " 0.5 " Ringer's solution	0.5 " " " " 0.5 " testicle extract	16.0 0.0	Strongly congestive and edematous, clearly defined No reaction

days after the last injection each animal was injected intracutaneously on one side with 1 cc. each of increasing dilutions of horse serum mixed with an equal volume of testicle extract. On the other side the same dilutions of horse serum to which an equal volume of Ringer's solution had been added were injected as controls. The records of the lesions as they appeared after 24 hours are given in Table IV. It should be noted that the lesions described as erythematous completely disappeared by the end of 2 days, while the hemorrhagic or necrotic ones healed slowly. The general characters of the reactions are illustrated in Figs. 1 to 4.

It will be seen that the results recorded for the Shwartzman phenomenon are essentially duplicated in the Arthus phenomenon. The more marked the dissemination of the injected material under the influence of the testicle factor the less intense is the severity of the reaction.

*The Effect of Testicle Extract on Infective Agents at High Dilutions*

The above experiments show that mild lesions produced by inanimate materials are suppressed or rendered barely detectable if they are spread through a large area. It seemed necessary to study more closely the action of testicle extract on infective agents which are known to be definitely enhanced in their infectivity by this extract. The following experiment is designed to test the effect on dilution of the infecting agents.

*Experiment.*—Three strains of staphylococcus with widely differing degrees of invasiveness for the skin of rabbits were selected for this test. Suspensions of these organisms were diluted progressively with water, so that they represented 1:200 to 1:20,000 of a 24 hour agar culture. 0.5 cc. of each dilution was mixed with 0.5 cc. of testicle extract and injected intracutaneously in one side of the rabbit. On the other side for control the injections were the same except that water was substituted for the extract.

The same procedure was carried out using vaccine virus as the infecting agent. The source of the virus was the standard testicle pulp of neuro virus described in previous publications. The degree of severity of the lesions as they developed during the period after inoculation is indicated by plus signs. In two of the tests here the spreading factor from an invasive strain of staphylococcus was substituted for testicle extract. The results are brought together in Table V, and the types of lesions are shown in Figs. 5 to 10.

TABLE IV  
*Effect of Testicle Extract on the Lesions Produced by the Intracutaneous Injection of Horse Serum in the Hypersensitive Rabbit (Arthus Phenomenon)*

Rabbit No.	Material injected	Area of lesions sq. cm.	Character of the lesions
1	1 cc. undiluted serum plus 1 cc. Ringer's solution " " " " " testicle extract	25.1 120.0	Strongly hemorrhagic and necrotic, clearly defined Moderately congestive, diffuse, flat. Central part hemorrhagic and necrotic
2	" " " " " Ringer's solution " " " " " testicle extract	31.1 82.8	Strongly hemorrhagic and necrotic, clearly defined Moderately congestive, diffuse, flat. A few hemor- rhagic spots
3	" serum diluted 50% " " " Ringer's solution " " " " " testicle extract	16.5 66.8	Strongly hemorrhagic and necrotic, clearly defined Erythematous, diffuse, flat
4	" " " " " Ringer's solution " " " " " testicle extract	16.8 70.7	Strongly congestive, clearly defined Moderately congestive, diffuse, flat
3	" " " " " 20% " " " Ringer's solution " " " " " testicle extract	9.1 66.8	Strongly hemorrhagic and necrotic, clearly defined Erythematous, diffuse, flat
4	" " " " " " " " Ringer's solution " " " " " testicle extract	12.9 70.0	Strongly congestive, clearly defined Erythematous, diffuse, flat
3	" " " " " 10% " " " Ringer's solution " " " " " testicle extract	8.4 25.1	Moderately hemorrhagic and necrotic, clearly defined Faintly erythematous, diffuse, flat
4	" " " " " " " " Ringer's solution " " " " " testicle extract	11.5 70.0	Strongly congestive, clearly defined Faintly erythematous, diffuse, flat



1	1 cc. serum diluted 10% plus 1 cc. Ringer's solution	16.0	Moderately hemorrhagic and necrotic, clearly defined
	" " " " " "	53.4	Faintly erythematous, diffuse, flat
2	" " " " " "	16.8	Strongly congestive, clearly defined
	" " " " " "	42.2	Faintly erythematous, diffuse, flat
3	" " " " 5% " "	7.0	Strongly hemorrhagic, clearly defined
	" " " " " "	20.0	Faintly erythematous, diffuse, flat
4	" " " " " "	10.8	Moderately congestive, clearly defined
	" " " " " "	27.0	Faintly erythematous, diffuse, flat
3	" " " " 2.5% " "	6.25	Erythematous, rather diffused
	" " " " " "	?	Almost unnoticeable erythema
4	" " " " " "	10.8	Erythematous, clearly defined
	" " " " " "	?	Almost unnoticeable erythema

TABLE V  
*Lesions Produced by Progressively Smaller Fractions of a 24 Hour Bacterial Culture or Standard Virus Pulp Injected Together with a Fixed Quantity of Distilled Water or Spreading Factor*

Fractions of culture or virus pulp	Distilled water					Testicle extract				
	1:200	1:1000	1:2000	1:10,000	1:20,000	1:200	1:1000	1:2000	1:10,000	1:20,000
Invasive staphylococcus.....	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
Moderately invasive staphylococcus.....	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Non-invasive staphylococcus.....	++	++	++	++	++	±	±	±	±	±
Vaccine virus.....	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
" *.....	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
" *.....	++	++	++	++	++	++	++	++	++	++

\* These two tests were carried out with a purified spreading factor from invasive staphylococcus (5).

An interesting contrast is shown in Table V between the action of testicle extract on a bacterium and a virus. With the bacteria, when the numbers injected fall below a certain level there is no longer an enhancement by testicle extract and there may actually be a definite inhibition of the manifestation of infection. On the other hand, within the limits of dilution of the vaccine virus, there is an invariable intensification of the lesion by the spreading factor from either testicle or an invasive strain of staphylococcus.

#### DISCUSSION

The experiments recorded above show that the lesions resulting from reactions between a normal or hypersensitive organism and certain inanimate agents (toxins and foreign sera) are spread through a much larger area if the agents are injected with testicle extract. This dissemination of the injected materials results in a considerable diminution in intensity of the induced lesion as compared with controls without testicle extract. What would be an intense reaction normally, if spread over a large area, might give the impression of a definite enhancement; but undoubtedly what has been gained in size has been lost in severity. The milder reactions may be to all intents and purposes completely suppressed if the inoculated agent is spread over a sufficiently large area. The conclusion seems justified that the capacity for injury of the agents used is not essentially modified by testicle extract.

A similar result was obtained with an organism of low virulence, a non-invasive strain of staphylococcus, when diluted somewhat to reduce the number of organisms injected with the testicle extract, as further with an invasive strain when highly diluted. The principal difference between the reaction to the inanimate agent and the living organism under the influence of testicle extract is that with larger numbers of organisms the resulting lesions are not only more extensive but more severe than in the controls, while the reaction to the inanimate agent loses in intensity what it gains in area. The spreading of the infectious agent through a large area does not have the same modifying effect on vaccine virus as on the staphylococcus. Here even in the extreme dilutions there seems to be a definite augmentation of severity of the lesions with the addition of the testicle

factor. Whether this represents a difference in behavior of viruses and bacteria in general, or merely a difference in the effective range of dilution, is being investigated.

It seems probable from these investigations that the reported enhancement by testicle extract of lesions induced by inanimate injurious agents is not in reality a true augmentation of the intensity of the reaction. While the sizes of the lesions under the influence of the spreading factor are undoubtedly larger, what has been gained in size has been lost in intensity. As far as our experiments go, true enhancement by the spreading factor is obtained only in the case of infectious agents.

#### SUMMARY

The lesions produced by the Shwartzman and Arthus phenomena, as well as those produced by bacterial toxin and foreign sera in the normal rabbit, are spread by testicle extract over a larger area than would normally take place. With this spreading of the lesions there is a definite reduction in their intensity.

Lesions produced by invasive strains of staphylococcus at high dilutions or non-invasive staphylococci at moderate dilutions are definitely lessened in severity or even suppressed by the spreading action of testicle extract. Virus lesions are consistently enhanced by the spreading factor regardless of the dilution.

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#### EXPLANATION OF PLATES

##### PLATE 23

FIG. 1. Rabbit 1 (right side). *a*, Arthus phenomenon lesion produced in the hypersensitive animal by the intracutaneous injection of 1 cc. of horse serum plus 1 cc. of Ringer's solution. 24 hours after injection. *b*, lesion produced by 0.1 cc. of horse serum plus 1.9 cc. of Ringer's solution. 24 hours after injection. *x*,

scar from the lesion produced by the intracutaneous injection of 1 cc. of horse serum 13 days before.

FIG. 2. Rabbit 1 (left side). *a*, lesion produced by the intracutaneous injection of 1 cc. of horse serum plus 1 cc. of bull testicle extract. 24 hours after injection. *b*, lesion produced by 0.1 cc. of horse serum plus 0.9 cc. of Ringer's solution plus 1 cc. of bull testicle extract. 24 hours after injection.

FIG. 3. Rabbit 2 (right side). *a, b, c, d, e*, decreasing Arthus phenomenon lesions produced by the intracutaneous injection in the hypersensitive animal of 0.5, 0.2, 0.1, 0.05, and 0.025 cc. respectively of horse serum diluted with Ringer's solution to a volume of 2 cc. 24 hours after injection. *x*, scar from the lesion produced by the intracutaneous injection of 1 cc. of horse serum 13 days before.

FIG. 4. Rabbit 2 (left side). *a, b, c, d, e*, effects produced by the same decreasing amounts of horse serum as in the other side, plus 1 cc. of bull testicle extract and Ringer's solution to a volume of 2 cc. 24 hours after injection.

## PLATE 24

FIG. 5. Rabbit 3 (left side). *a, b, c, d, e*, decreasing lesions produced by the intracutaneous injection of 1:200, 1:1000, 1:2000, 1:10,000, and 1:20,000 respectively of a 24 hour agar culture of a non-invasive strain of staphylococcus, each injection consisting of 0.5 cc. of the bacterial suspension plus 0.5 cc. of water. 24 hours after injection.

FIG. 6. Rabbit 3 (right side). *a, b, c, d, e*, effect produced by the same decreasing amounts of non-invasive staphylococcus plus 0.5 cc. of bull testicle extract. Notice the absence of lesions in spots *d* and *e* and the mild lesions in the other spots. 24 hours after injection.

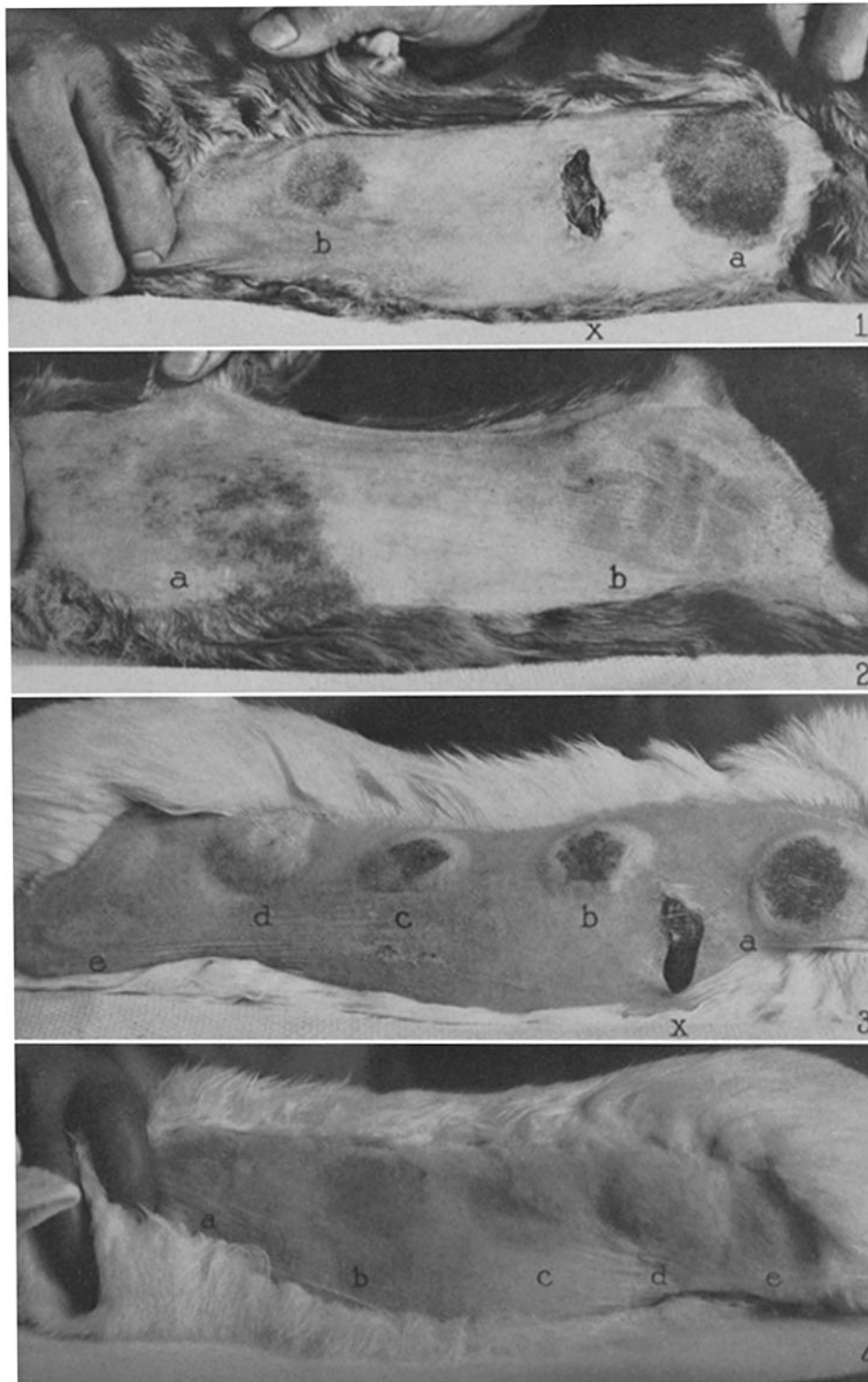
FIG. 7. Rabbit 4 (right side). *a, b, c, d, e*, decreasing lesions produced by the intracutaneous injection of 1:200, 1:1000, 1:2000, 1:10,000, and 1:20,000 of a 24 hour agar culture of a very invasive strain of staphylococcus, each injection consisting of 0.5 cc. of bacterial suspension plus 0.5 cc. of water. 24 hours after injection.

FIG. 8. Rabbit 4 (left side). *a, b, c, d, e*, lesions produced by the same decreasing amounts of the very invasive staphylococcus plus 0.5 cc. of bull testicle extract. Notice the still slight enhancement in the spots *a* and *b* and the marked inhibition in the other spots. 24 hours after injection.

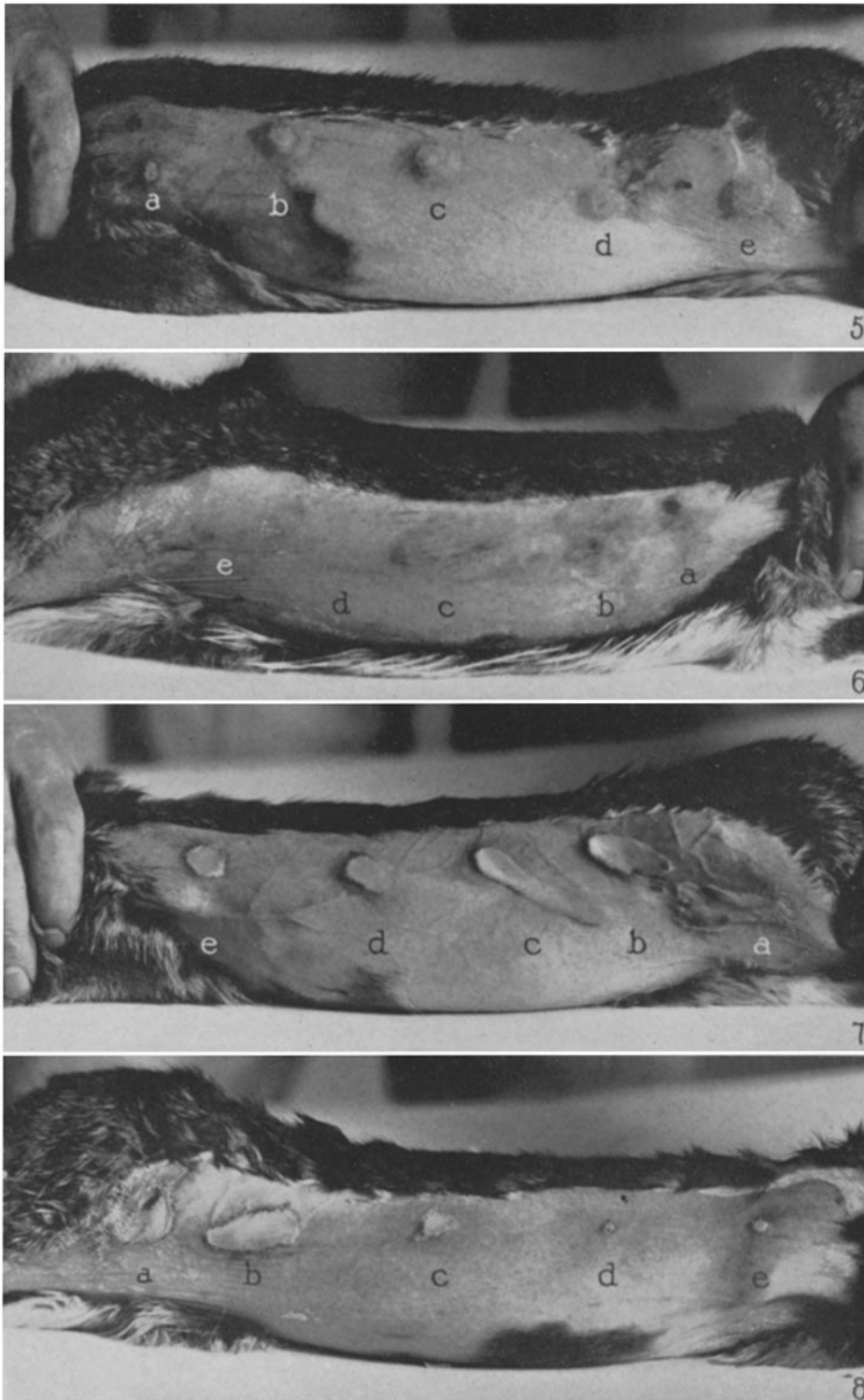
## PLATE 25

FIG. 9. Rabbit 5 (left side). *a, b, c, d, e*, decreasing lesions produced by the intracutaneous injection of 1:200, 1:1000, 1:2000, 1:10,000, and 1:20,000 of a standard vaccine virus pulp, each injection consisting of 0.5 cc. of the virus suspension plus 0.5 cc. of Ringer's solution. 5 days after inoculation.

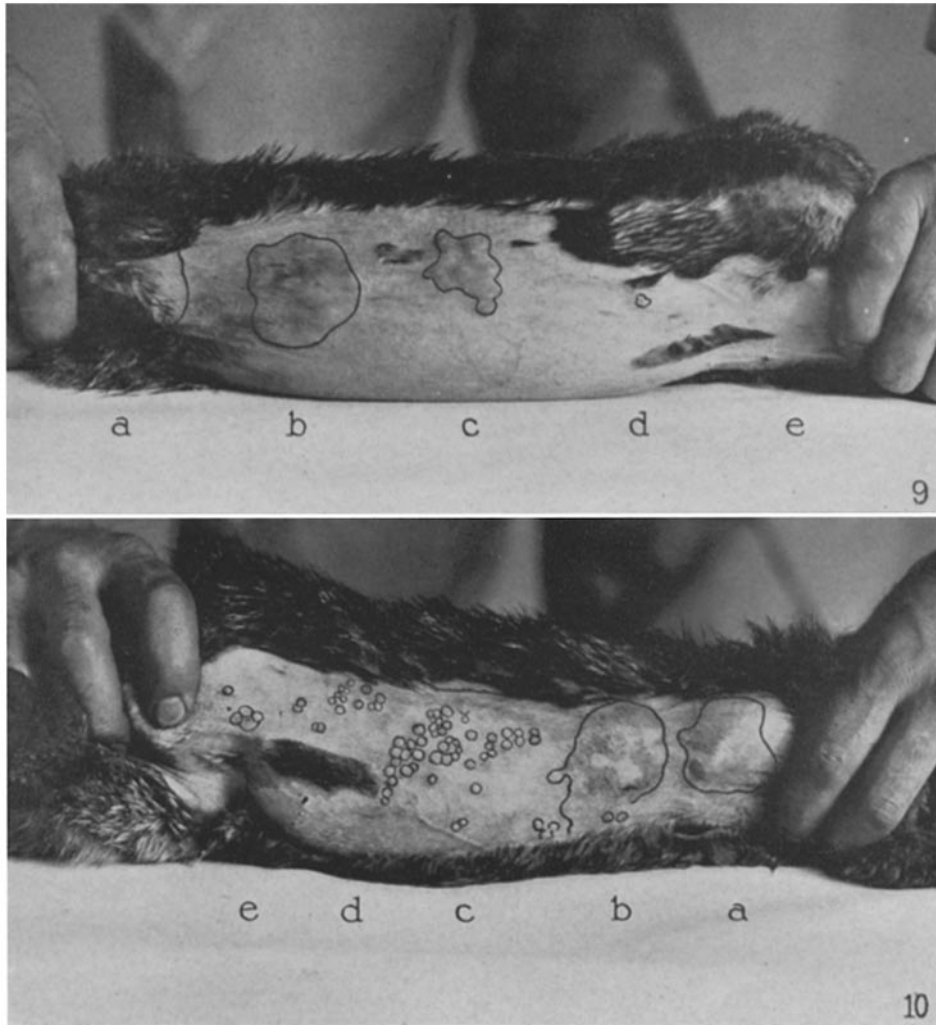
FIG. 10. Rabbit 5 (right side). *a, b, c, d, e*, lesions produced by the same decreasing amounts of vaccine virus plus 0.5 cc. of bull testicle extract. 5 days after injection. Notice the marked enhancement of the lesions at all dilutions of the virus.



(Duran-Reynals: Effect of testicle extract on toxins)



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