# THE EFFECT OF EXTRACTS OF CERTAIN ORGANS FROM NORMAL AND IMMUNIZED ANIMALS ON THE INFECTING POWER OF VACCINE VIRUS

By F. DURAN-REYNALS, M.D.

*(From the Laboratories of The Rockefeller Institute for Medical Research)*

Plates 14 and 15

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In a previous paper (1) we reported that when the agent of Chicken Tumor I was brought in contact with finely ground muscle from susceptible animals it was bound so that both the supernatant fluid and the muscle tissue became partially or completely inactive when injected into chickens. The muscle of non-susceptible animals such as pigeon and rabbit, or even organs such as liver, brain and kidney of susceptible chickens do not affect the activity of the agent.

The present study was undertaken in order to determine whether a similar effect could be demonstrated for a typical representative of the group of the so-called filter-passing viruses, such as vaccine virus. As laboratory animals are more or less susceptible to vaccine virus, for the refractive group it has been necessary to use artificially immunized rabbits, although it is realized that subsequent work may show that the comparison is unjustified.[1]

The fact that tissues of immunized animals inactivate the virus *in vitro* has already been shown. Thus Levaditi and Nicolau (4) report that vaccine virus and herpes virus were "destroyed" by the brain tissue from immunized animals. Tessier, Gastinel and Reilly (5) using a small number of animals confirmed the above result with herpes virus and further determined that the brain and serum from naturally immune rabbits and non-susceptible dogs are devoid of action on the virus.

[1] A preliminary note on the subject has already been published and some of its points have been duplicated on the staphylococcus (2), (3).

## *Methods and Materials*

*Vaccine Virus.*—Three different strains of virus have been used, namely, the Noguchi testicular vaccine virus, the Levaditi neurovaccine, and the cow glycerinized dermovirus of the Department of Health, City of New York.[2]

The specimen of testicular virus was prepared by injecting 0.5 cc. of vaccine emulsion mixed with an equal amount of Ringer's solution into both testicles of a rabbit. 5 days later, when the resultant orchitis was at its height, the animal was killed, and the testicles removed aseptically. They were ground thoroughly with sand together with 25 cc. of glycerol and 25 cc. of Ringer's solution. This emulsion was distributed in tubes, covered with a layer of sterile vaseline, and kept in the ice-box.

The specimen of neurovirus was prepared by injecting about 0.2 cc. of a heavy suspension of infected brain tissue into the brain of a rabbit. After 5 days the brain was removed aseptically and treated in the same manner as the testicular virus. Dilutions, usually at 1:50, were made immediately before each experiment with Ringer's solution.

The animals were carefully shaved, so as to avoid any injury of the skin, and generally a test injection was made intracutaneously on one side, the opposite side being used as control in order to avoid the disturbing fact of the individual differences in susceptibility.

*Inactivation of Testicular Virus by Immune Brain Extract.*—As mentioned above, Levaditi has shown that an extract of the brain of an immune animal neutralized the neurovaccine. This experiment has been repeated, substituting Noguchi's testicular stain for the neurovirus.

The brains of two rabbits immune to the testicular virus were used, controlled by the brains from two normal rabbits. A half of each brain was ground and mixed with 2 to 3 cc. of a 1:10 virus dilution. The mixture was kept for 5 hours at room temperature in one instance and in the other for 3 hours at 37° and then overnight in the ice-box. The mixtures were then centrifuged and the supernatant fluids and pulps injected separately into normal rabbits. The results are shown in Table I.

TABLE I

| Exper. | Lesions produced by supernat. fluids | | Lesions produced by pulps | |
|---|---|---|---|---|
| | Immune brain | Normal brain | Immune brain | Normal brain |
| I | 0.5 cc. ± | + | 0.1 cc. − | + |
| | 0.5 cc. ± | + | 0.1 cc. ± | ± |
| | 0.5 cc. − | + | 0.1 cc. ++ | ++++ |
| II | 0.5 cc. − | ± | 0.1 cc. + | + |
| | 0.5 cc. − | − | 0.1 cc. + | + |

[2] We wish to acknowledge our indebtedness to Dr. T. M. Rivers for supplying the original strains of testicle and neurovirus.

*Inactivation of Testicular Virus by Immune Testicle Tissue.*—The same experiment was repeated using testicle instead of brain tissues.

Immune rabbits from 1 to 3 months after the immunizing injection of testicle or neurovirus were used. The testicles were removed and ground without sand, and measured amounts of the resultant pulps were mixed with equal volumes of 1:50 testicle virus dilution. The length of contact was 3 hours at room temperature in one instance and from 1 to 3 hours at room temperature and overnight in the ice-box for the rest of the experiments. The mixtures were then centrifuged and the supernatant fluids injected into normal rabbits. The same procedures were carried out with normal testicles in the control experiments.

The results are summarized in Table II.

TABLE II

| Exp. No. | Lesions by supernat. fluids from: | | Lesion by pulps from: | |
|---|---|---|---|---|
| | Immune testicle | Normal testicle | Immune | Normal |
| 3 | 0.2 cc. − | +++ | 0.2 cc. − | + |
| | 0.5 cc. ± | ++++ | | |
| 4 | 0.2 cc. ± | +++ | 0.1 cc. − | +++ |
| | 0.5 cc. ± | +++ | 0.1 cc. − | +++ |
| 5 | 0.2 cc. − | +++ | Scarification − | + |
| | Scarification − | ++ | | |
| 6 | 0.5 cc. − | ++ | No test | No test |
| 7 | 0.2 cc. + | ++ | 0.1 cc. + | ++ |
| 8 | 0.3 cc. − | ++ | 0.1 cc. − | ++ |
| 9 | 0.5 cc. − | +++ | 0.1 cc. − | ++ |
| 10 | 0.5 cc. − | +++ | 0.2 cc. − | ± |
| 11 | 0.5 cc. − | +++ | 0.2 cc. + | +++ |
| | 0.5 cc. − | +++ | 0.2 cc. + | ++ |

It is evident from these tests that the brain and the testicle from immune animals inactivate vaccine virus, the latter tissue being the

more active. The inactivation takes place quickly, as a contact of 3 hours reduced the activity of the virus dilution as completely as a longer contact.[3]

*Perfusion Experiments.*—It is known that the serum from an immune animal exhibits so-called viricidal action on vaccine virus. Hence the results obtained so far might be ascribed to the inactivating power of the blood contained in the tissue and not the tissues themselves. To check this point the following experiments were performed.

A 3 months immune rabbit was perfused under anesthesia with Ringer's solution and sodium citrate through the abdominal aorta and the vena cava until the testicles became perfectly white. The same procedure was carried out on a normal rabbit and the above experiments were repeated with this material. The mixture of testicle and virus was kept at room temperature for 3 hours and then overnight in the ice-box. A similar experiment was carried out with neurovirus. The results are given in Table III.

TABLE III

| Virus | Lesion by supernatant fluids | | Lesion by pulps | |
|---|---|---|---|---|
| | Immune testicle | Normal testicle | Immune testicle | Normal testicle |
| Testicle | 1 cc. − | ++++ | 0.1 cc. ± | ++ |
| Neuro- | 1 cc. + | Very extensive lesion | 0.1 cc. + | ++++ |

It appears that the lesions from immune animals possess an inactivating power as well as the serum.

*Speed of Reaction between Vaccine Virus and Immune Organs.*—A

[3] The question of whether or not the testicle tissue from suscepticle animals fixes the virus was answered by a set of experiments in which normal rabbits were injected with testicle emulsions immediately after the mixtures were made and again after some hours of contact. As controls the same amount of virus dilutions as those contained in the mixtures was also injected at the same time. As there was no difference in the extent of the lesions produced it may be concluded that there was no fixation of the virus by normal testicle tissue. That there was a mechanical retention or adsorption of the virus by the tissue was shown by the fact that washing of the tissue after contact did not materially reduce the activity of the material when injected into animals.

further attempt was made to determine the speed of reaction and its further progress during the time of contact.

3 cc. of ground testicle tissue from a 35 day immune rabbit was mixed with an equal volume of a 1:50 testicular vaccine dilution. Mixtures were immediately centrifuged and a sample of the supernatant fluids injected intrademally into a normal rabbit. The remaining fluid and tissue were mixed and allowed to remain in contact for 5 hours at room temperature and then for 17 hours in the ice-box. The mixture was again centrifuged and another sample of the supernatant fluid was tested on the same rabbit. A similar experiment was carried out with neurovirus. The results are given in Table IV.

TABLE IV

| Virus | Lesions produced by supernatant fluids immediately after mixing | | Lesion by supernatant fluids and pulps after 22 hours of contact | |
|---|---|---|---|---|
| | Immune testicle | Normal testicle | Immune testicle | Normal testicle |
| Testicular | 0.5 cc. − | +++ | 0.25 cc. +<br>0.50 cc. +<br>0.10 cc. ± | +++<br>++++<br>+++ |
| Neuro- | 0.5 cc. + | Very large lesion | 0.25 cc. +<br>0.50 cc. + | ++++<br>++++ |

These tests indicate that the inactivation of the virus by the testicle from immune animals is accomplished very quickly and is not notably increased by further contact.

The possibility that the virus was inactivated but not destroyed by the contact with tissues from immune animals was tested. The mixtures subjected to tryptic digestion, desiccation or treatment with solutions of different pH failed to release the virus in active form.

*Enhancement of Neurovirus by Normal Testicular Extract.*—It will be noted that in the above experiment the neurovirus was inactivated by the testicular virus. On the other hand, the normal testicular extracts, which are devoid of appreciable effect on the testicular strain, gave evidence of an enormous augmentation in the skin lesions pro-

duced by the neurovirus. This observation was confirmed by the experiments which are summarized in Table V.

TABLE V

| Lesions produced by the supernatant fluid from: | | Lesions produced by the pulps from: | |
|---|---|---|---|
| Immune testicle | Normal testicle | Immune testicle | Normal testicle |
| 1. 0.2 cc. + | Very large lesion | 0.2 cc. + | Very large lesion |
| 2. 0.2 cc. ++ | Very large lesion | 0.2 cc. + | ++++ |
| 3. 0.2 cc. + | Very large lesion | 0.2 cc. + | ++ |

The lesions were much more hemorrhagic and necrotic and spread over a much greater area than those produced by the virus alone. The two rabbits injected with the supernatant fluid from the mixture of normal testicular extract plus neurovirus became very sick, lost considerable weight, and died 6 and 7 days respectively after the injection. One of the rabbits injected with the pulp showed also a large lesion and became sick but eventually recovered. The other rabbits showed the usual vaccinal eruption.

*Detailed Study of the Enhancement of the Neurovaccine Virus by Testicle Extracts.*—The nature of the lesions obtained in the foregoing set of experiments warranted a further and careful study of the power shown by normal testicle extracts in enhancing to such a high degree the infecting power of the Levaditi neurovirus.

The technique at first was the same as in the foregoing experiments, and pulps as well as supernatant fluids were injected. Later on the procedure was simplified by grinding the testicle together with its volume of Ringer's solution and centrifuging immediately. Only the cloudy supernatant fluid was used. About 0.5 cc. of this was mixed with 0.25 cc. of a 1:50 dilution of infected tissue emulsion immediately before the injection.

With the supernatant fluid the enhancement has been observed in practically all of the 80 tests carried out. In 2 or 3 instances where the enhancement was doubtful or negative, the effect was traced to the use of a feeble virus or atrophic testicles. The injection of the pulps in the amounts used did not give constant results, as in 5 out of 11 instances there was no enhancement, and in 2 cases only doubtful enhancement.

Only in a few instances did the virus-testicle mixture give an earlier lesion than the virus alone. Generally on the 2nd or 3rd day evidence of the infection became detectable on both sides, but the lesion was very much less extensive in the control side. The lesions produced by the neurovirus alone generally reached their maximum spread by the 4th or 5th day and presented the usual picture of a more or less hemorrhagic localized eruption. On the other hand the lesions produced by the virus plus testicle extract generally continued to spread till the 6th or 7th day, by which time they had extended throughout the flank and even over the abdomen. The skin appeared thick and very red or violaceous in color, and it was often covered with blisters. Areas of necrosis often appeared in the middle of the lesion and sometimes the whole of the skin area was necrosed. There was generally a marked oedema in the neighbouring regions.

General symptoms in the rabbits injected intradermally with the testicle-neurovirus mixture became pronounced. The temperature reached 105°F. and sometimes 106° and 107°. In most cases hypothermia followed in the days immediately before death. There was considerable loss in weight—sometimes more than 600 gm.—and most of the animals developed severe diarrhea and signs of pulmonary disease. Conjunctivitis was observed in a few instances.

Death occurred in about 25 per cent of cases. The most striking findings at postmortem examination were a double hemorrhagic lobar pneumonia with sometimes widespread pulmonary abscesses and severe glomerulo-nephritis. Lymph nodes in the vicinity of the lesion were enlarged and congested and the testicles were sometimes congested. The details of the histological lesions of this generalized vaccinal infection will be reported in a later paper. Typical alterations were found in the ovaries, tesicles, suprarenals, lungs, etc., and vaccine virus was easily recovered from these and other organs independently of its presence in the blood.

In other animals purposely killed at the height of the disease, more or less pronounced lesions were also found in the lungs. The general clinical and histo-pathological picture of disease caused by the vaccine virus plus testicle extract is that of the usual infection by the virus alone, but extraordinarily enhanced.

*The Influence of Other Organ Extracts on Neurovirus Infection.*—It was obviously interesting to know how organs other than testicle behave when injected along with neurovirus.

The same technique was employed, the supernatant fluids only being used. The mixture of virus and tissue extracts or serum was made immediately before injection, in the ratio of 0.25 cc. of a 1:50 dilution of the virus emulsion to 0.50 cc. of the organ or tissue extract.

The results are summarized in Table VI.

TABLE VI

| Organ extract injected with neurovirus | No. of tests | No. enhanced | No. unmodified | No. decreased | No. suppressed |
|---|---|---|---|---|---|
| Testicle | 80 | 78 | 2 | 0 | 0 |
| Kidney | 6 | 6 | 0 | 0 | 0 |
| Skin | 2 | 2 | 0 | 0 | 0 |
| Suprarenal | 4 | 0 | 4 | 0 | 0 |
| Blood (whole) | 3 | 0 | 1 | 2 | 0 |
| Serum | 10 | 0 | 5 | 5 | 0 |
| Bone marrow | 8 | 0 | 5 | 3 | 0 |
| Lymph nodes | 2 | 0 | 2 | 0 | 0 |
| Spleen | 24 | 0 | 8 | 14 | 2 |

In addition to the above, one test each was made with several other tissues. The indications from these are that liver, brain and placenta enhance the infection, while muscle, retina and the whole embryo are without effect.

From the study of the results we are able to classify the organs or tissues as regards their influence on vaccinal infection as follows: (1) organs that always enhance, such as testicle, kidney, etc.; (2) organs which neither enhance nor interfere, as, probably, muscle, suprarenal, etc.; (3) organs which never enhance and very often interfere with and even suppress the infecting power of the neurovirus, such as spleen, blood, etc.

Among the organs endowed with an enhancing power the testicle is by far the strongest, whereas the spleen seems most active among the organs which interfere with the infection. It is interesting to note that the inhibiting power of the spleen is quickly lost by dilution, the pulps being the most effective, whereas the enhancing power of the testicle, as will be seen later, is unaffected by high dilutions.

*Enhancement of the Dermovaccine Virus by Testicle Extracts.*—In view of the fact that the enhancement of the vaccine virus in the skin was positive with the neuro- and negative with the Noguchi testicular virus, it was desirable to determine whether the testicular extract was capable of enhancing the activity of the usual cow dermovirus.

Rabbit testicle extract was prepared in the usual way, and 0.5 cc. was injected intradermally together with the entire content of a tube of dermovirus (a human dose) into the left side of two rabbits. The right side was injected with the virus

alone with Ringer's solution. The left side showed lesions 5 times larger than the right side, but these lesions were milder than those caused by the neurovirus or the testicle virus.

*Enhancement of the Noguchi Testicle Virus.*—That the feebler activity of the Noguchi testicle virus is not responsible for its lack of activation by testicle extract is clearly shown by the foregoing experiment where a still feebler virus, the cow virus, is definitely enhanced by the same extract.

Kidney extracts proved to be effective in one experiment in enhancing slightly but definitely the testicle virus infection in the skin. In another experiment testicle virus was injected into the brain of a rabbit which was killed 5 days afterwards, and this brain, used as neurovirus, proved to be definitely enhanced by testicle extract. The lesions obtained were the usual mild lesions produced by the testicle virus. On the other hand, the neurovirus after growth in the testicle lost none of its virulence and was still actively enhanced by testicle extracts when injected intradermally. It is therefore obvious that enhancement of testicle virus by organ extracts is possible under certain conditions.

*Enhancement of Neuro- and Testicle Virus by Testicle and Kidney Extracts when Injected into the Testicles.*—These experiments were performed in order to determine the effect of the enhancement in organs other than skin, namely, testicles.

The general procedure in preparing the material does not differ from that used in the skin experiments. The right testicle was used for injections and the left used for comparison. The results of 4 experiments on 16 rabbits are summarized in Table VII.

TABLE VII

| Lesion produced by: | | | Lesion produced by | | |
|---|---|---|---|---|---|
| | Testicle virus plus organ ext. | Testicle virus plus Ringer's | | Neurovirus plus organ ext. | Neurovirus plus Ringer's |
| Testicle..... | + | ++ | Testicle..... | +++ | +++ |
| Kidney..... | +++ | −[4] | Testicle..... | ++++ | +++ |
| Testicle..... | +++ | | Testicle..... | ++++ | |
| Kidney..... | +++ | − | Kidney..... | ± | + |
| Kidney..... | + | | Kidney..... | ++++ | |

[4] This minus sign does not show complete absence of orchitis but simply the complete absence of any gross inflammation. That infection had taken place is shown by the fact that an active virus may be recovered.

From the study of this table we can draw the conclusions that, in spite of some irregularity of both neuro- and testicle virus in giving rise to definite orchitis in rabbits, both can be enhanced by either testicle or kidney extract when injected into rabbit testicles.

*Experiments on the Nature of the Enhancement.*—The enhancement is not species specific, as the tests show that rabbits injected with neurovirus plus rat or guinea pig testicle extract, prepared and used as the rabbit extract, exhibit typical enhanced lesions. All animals injected became sick and some died on about the 8th day, with the usual general signs especially in lung and kidney. The age of the animal from which the organs for the extracts are taken, as well as the age of the injected animal, has no influence on the phenomenon of enhancement. Animals solidly immune to both neuro- and testicle virus are as resistant to infections by the virus plus testicle extract as by the virus alone. Slightly autolyzed extracts are endowed with the same power as extracts recently obtained.

The virus does not seem to be modified in its virulence, for strains secured from the enhanced lesions are no more highly infective than those secured from the ordinary lesion. That, at least, the action of the extracts is directed toward the cell is shown by the occasional observation that some rabbits having pronounced lesions from pure neurovirus showed a typical vaccinal eruption in spots injected only with testicle extract. This suggested an experiment in which the shaved skin of a rabbit received in three different spots testicle extract,[5] and at the same time 1 cc. of neurovirus diluted with 20 cc. of Ringer's solution was injected into the ear vein. Typical lesions developed at the three injected spots and the animal died on the 4th day, showing that the blood virus localized only in the sensitized spots. After the injection of testicle extract the cells remained sensitized for a certain length of time. This was shown by an experiment in which 5 rabbits were injected intradermaly with 0.5 cc. of the extract while in the course of the following days 0.25 cc. of a 1:50 dilution of the virus was injected in the same spots. Another spot on the skin was injected with the virus alone in each case as a control. Enhanced lesions were

[5] The extract used in this experiment was kept 24 hours at room temperature and 24 more in the ice-box. Two similar experiments dealing with testicle virus gave negative results.

obtained up to the 3rd day, whereas later the lesions were not different from those obtained in the control spots, or the enhancement was doubtful. The virus used was of moderate strength, so that perhaps a longer sensitization would have been observed with a more active virus.

*Preliminary Experiments on the Physical and Chemical Nature of the Enhancing Substance.*—As the result of a number of preliminary experiments some of the properties of the enhancing substance have been determined. Dilutions of the supernatant fluids of testicle extracts, as high as 1:160 are almost as active in their enhancing power as the full strength extracts. Berkefeld filtrates of the extracts contain the enhancing substance in considerable amounts. Exposure to 100°C. for 3 minutes completely destroys the activity of the extracts. The precipitate resulting from the acidification of a Berkefeld filtrate of testicle extract contains practically all of the enhancing substance. This precipitate gives uniformly a strong Feulgen reaction, indicating a predominance of nucleoprotein elements.

## DISCUSSION

The experiments reported here show that the neuro-, testicular and cow strains of vaccine virus are not only uninjured by contact *in vitro* with sensitive tissues from susceptible animals, but their infectivity is enhanced to an extraordinary degree by such contact. This finding is in strong contrast to the results obtained when a chicken tumor agent is subjected to similar treatment. The fact that one agent is either bound or inactivated by the constituents of the more susceptible tissue while another has its activity greatly enhanced suggests a fundamental difference in the mechanism involved in the action of the two and tends to separate them into different classes. It is known that certain enzymes form a union with the specific substance on which they act and this is supposed to be true in general for the enzyme-like agents. Whether or not the virus group is uniformly unaffected by contact *in vitro* with tissues of susceptible animals, or may have their activity enhanced by such tissues, remains to be determined. In the instance of the vaccine virus the evidence is conclusive.

The result of contact of vaccine virus with tissue extracts from immune animals and the chicken tumor agent with tissues from susceptible fowls is the same, namely, inactivation in both instances. The fact that vaccine virus is inactivated particularly by the sera from immune animals is well known and is generally considered to be the result of a real destruction of the virus. But the fact that the reaction between the virus and the immune tissue extract reaches its maximum almost immediately after the contact is effected and does not progress, and furthermore, because of the similarity between this effect and the inactivation of the chicken tumor agent by sensitive tissues, seemed to indicate that the action was not a destruction of the virus. The recent work of Andrews (6) has shown that the active virus may be recovered after contact with immune serum even when the serum is used in great excess. Furthermore Long and Olitsky (7) have shown that active vaccine virus may be recovered by cataphoresis from the testicle of immune animals many months after the disappearance of all active lesions. Hence it seems that the virus is not necessarily destroyed by the immune substance.

The most active of the tissues in the augmentation of the infectivity of the vaccine virus is the testicle. It is of interest to note that this organ is not only the most sensitive tissue to direct inoculation but is with the ovary the site where, in absence of previous irritation, the vaccine virus localizes most abundantly after intravenous inoculation.

The mechanism of the enhancement is not clear. The fact that the virus injected into an area of skin some hours or days after the tissue extract has been injected into the same area, results in an enhanced lesion, suggests that the effect of the extract is on the host cells, rendering them more susceptible to the virus, rather than on the virus direct. This interpretation is further strengthened by the observation that virus injected intravenously localizes most readily in areas of skin previously injected with testicle extract, and the lesions resulting are very extensive. It may well be that the extracts act primarily as a stimulus to cell division, thus increasing the number of young cells which are supposed to be more susceptible to the virus effect than older cells.

Aside from the organs or tissues whose extracts enhance the activity of vaccine virus there are others which frankly interfere with the infec-

tion. Among these latter the spleen pulp shows greatest inhibiting effects. In this connection evidence associating lymphoid reaction with the inhibition of tissue growth is recalled. But regardless of the explanation of the phenomenon described it seems that a basic difference has been established between the behavior of the filterable agent of the chicken tumor and a typical member of the so-called filter-passing viruses.

## SUMMARY

Brain and testicle tissue from immune rabbits brought in contact with the Levaditi or Noguchi strains of vaccine virus will fix or inactivate the virus. Extracts of normal testicles from susceptible animals enhance to an extraordinary degree the infectivity of both the neuro- and dermal strains of vaccine virus. The Noguchi virus is not affected by testicle extracts when injected into the skin, but kidney extract has a definite enhancing power on the strain when injected into either skin or testicle.

The effect of tissue extracts seems to be on the cells of the host rather than on the virus. This is indicated by the fact that virus injected intravenously localizes most readily in an area of skin previously injected with testicle extract. Furthermore an enhanced lesion results if the virus is injected into an area as long as 3 days after the area has been injected with testicle extract.

The augmenting substance of the tissue extracts is little affected by high dilutions, passes through a Berkefeld V candle and is carried down with the proteins precipitated by weak acids.

Rabbits with enhanced lesions show general symptoms and about 25 per cent die with generalized vaccinia.

Kidney, and probably skin, brain and liver extracts possess enhancing properties, but to a much less degree than the testicle. On the other hand, spleen, blood and probably lymph nodes and bone marrow not only fail to produce enhancement, but actually restrain or even suppress entirely the vaccinal skin infection.

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## EXPLANATION OF PLATES

### PLATE 14

FIG. 1. (Rabbit 160, right side). Lesion produced by the intradermal injection 4 days before of 0.25 cc. of a 1:50 dilution of neurovirus plus 0.50 cc. of the precipitate obtained by the addition of acid to the Berkefeld and concentrated testicle extract.

FIG. 2. (Rabbit 160, left side). Control. Lesion produced by 0.25 cc. of the virus dilution plus 0.50 cc. of Ringer's. Note in the lower abdomen the oedema from the right side lesion.

FIG. 3. (Rabbit 163, right side).

1. Lesion produced by the injection 5 days before of 0.25 cc. of a 1:50 dilution of neurovirus plus 0.50 cc. of testicle extract.

2. Lesion produced by 0.25 cc. of the virus dilution plus 0.45 cc. of spleen extract.

FIG. 4. (Rabbit 163, left side). Control. Lesion produced by 0.25 cc. of a 1:50 dilution of virus plus 0.50 cc. of Ringer's.

### PLATE 15

FIG. 5. (Rabbit 97, right side).

1. Lesion produced by the injection 8 days before of 0.25 cc. of a 1:50 virus dilution plus 0.50 cc. of testicle extract.

2. Lesion produced by the injection 8 days before of 0.25 cc. of a 1:100 virus dilution plus 0.50 cc. of testicle extract.

3. Lesion produced by 0.25 cc. of a 1:100 dilution of neurovirus plus 0.50 cc. of kidney extract.

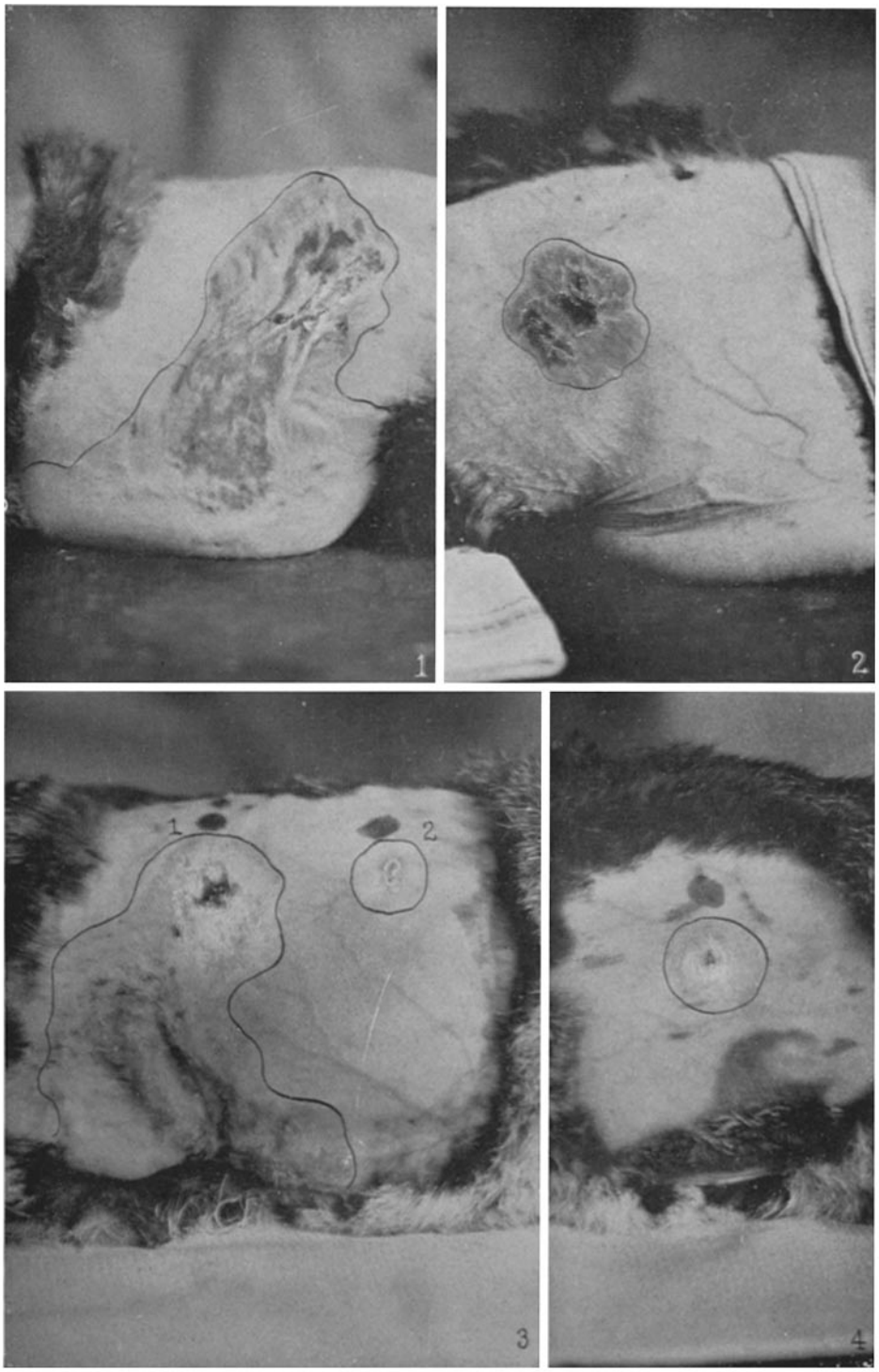
FIG. 6. (Rabbit 97, left side). Control.

1. Lesion produced by the injection 8 days before of 0.25 cc. of a 1:50 neurovirus dilution plus 0.50 cc. of Ringer's.

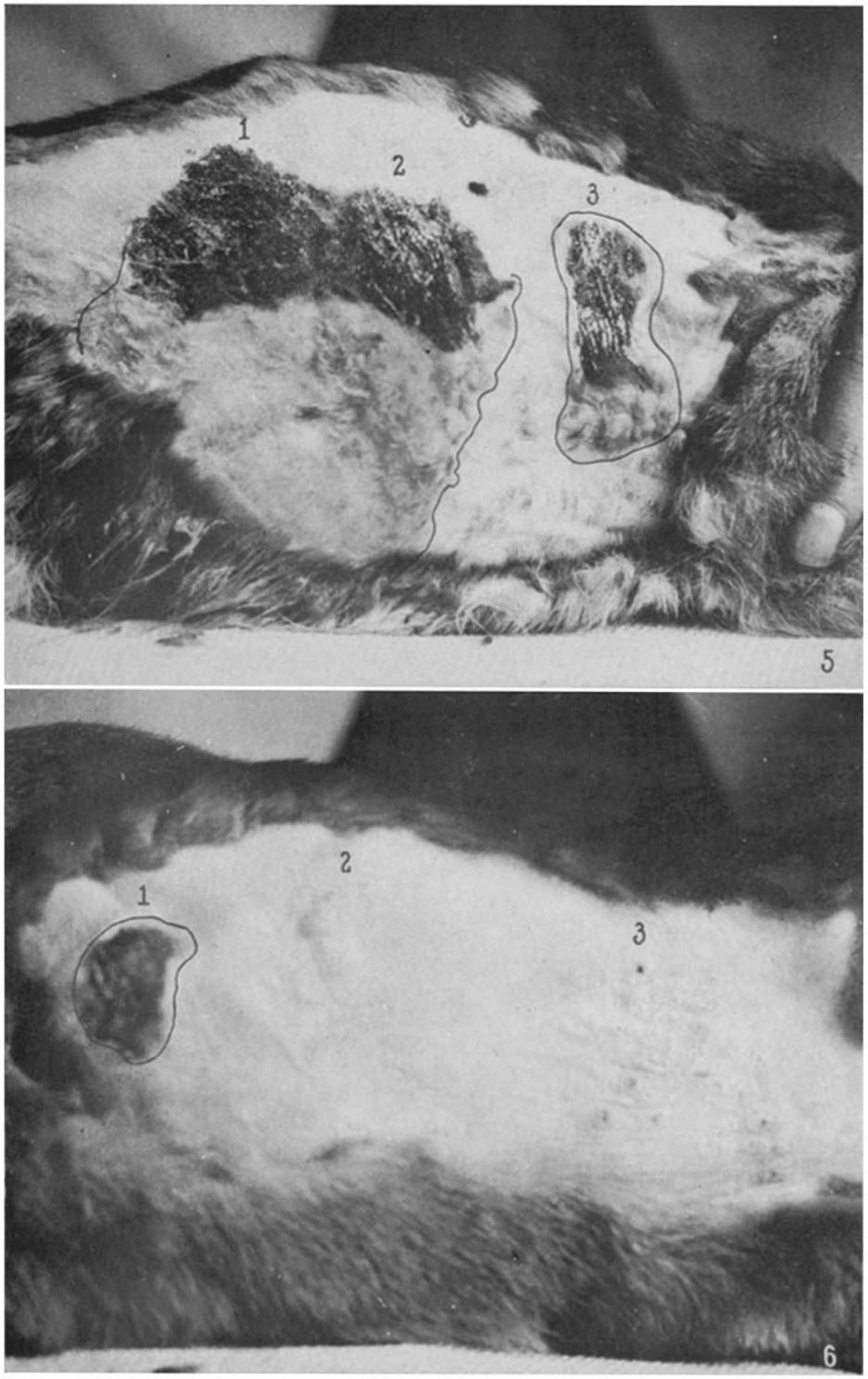
2. Spot injected with 0.50 cc. of kidney extract alone.

3. Spot injected with 0.50 cc. of testicle extract alone. Note the secondary localization of the virus.

(The rabbit died 2 days after the photographs were taken.)




(Duran-Reynals: Infecting power of vaccine virus)




(Duran-Reynals: Infecting power of vaccine virus)