STUDIES ON VARICELLA.

The Susceptibility of Rabbits to the Virus of Varicella.

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PLATES 38 TO 40.

In spite of the ignorance regarding the etiological agents concerned in the contagious diseases of children, it has been possible, through the careful clinical observations of such men as Sydenham, to differentiate one from the other. Notwithstanding this wide clinical knowledge, it is still impossible to control the spread of these diseases, or to arrest their course once they have established themselves in susceptible individuals. It is true that some progress has been made in the prevention of measles, scarlet fever, and chicken-pox by the introduction of the method of injecting serum from convalescent patients into exposed individuals. Attempts have also been made to prevent chicken-pox by vaccination with fresh vesicle lymph, and while a few workers have reported positive results with this method, the majority of investigators have not succeeded. It is evident that these procedures involve objectionable features which would detract from the usefulness of the methods were an attempt made to apply them widely. To develop better methods of prevention, it is highly desirable to transmit the diseases to animals in such a way as to obtain a concentrated virus similar to vaccine virus or the virus of rabies.

Varicella is usually considered a mild disorder, but, nevertheless, for many reasons, it has appeared to be a suitable one on which to begin study.

In the first place, chicken-pox, in spite of its mildness, ranks high among the infectious diseases as a cause of loss of time from schools, and is very troublesome in institutions, such as orphan asylums and hospitals for children. Also, knowl-

edge concerning chicken-pox would probably be directly applicable to the more severe disease, smallpox. Although this disease no longer prevails to the wide extent that it did in former centuries, nevertheless, it is still common in certain sections of the United States. According to Low (1), for the last 20 years there has been an average of 20,000 to 30,000 cases of smallpox every year. Recently, in 1 year, over 94,000 cases (2) were reported. Usually it is not difficult to differentiate smallpox from chicken-pox, but there are instances when it is not easy, and mistakes undoubtedly occur. Although Paul (3), Tièche (4), Force and Beckwith (5), Salmon (6), and Paschen (7) have introduced certain laboratory methods of aid in diagnosis, these are not entirely satisfactory. Furthermore, in chicken-pox there is a characteristic skin lesion which may serve as a mark of identification in case the disease were produced in animals. Although the recent work on herpes and lethargic encephalitis has aroused great interest in all diseases in which vesicular lesions on the skin are present, relatively little experimental work has been done on varicella.

There are other diseases, such as sheep-pox, vaccina, smallpox, alastrim, herpes zoster and symptomatic herpes, with which it is possible to compare chicken-pox. Certain of these diseases have been grouped under such general terms as "ectodermoses neurotropes" by certain investigators, for instance, Levaditi and Nicolau (8) and Lipschütz (9). From the numerous studies on these closely related diseases many facts are available which may be of aid in work undertaken with varicella. Upon first thought there seems to be little connection between sheep-pox and lethargic encephalitis in man. On considering all these diseases in a series, however, (Table I), studying the clinical relations existing between them and taking into consideration the results of the experimental studies which have been made, the two just mentioned do not seem so far removed from each other.

It is now generally believed that chicken-pox and smallpox are distinct and different diseases. This has not always been the case, however, and as late as the middle of the 19th century Hebra (10) taught that they were identical. Even at the present time there is a difference of opinion in regard to the relation of smallpox and varioloid to alastrim on the one hand, and of chicken-pox to alastrim on the other. Von Bókay's (11) paper on the relation of chicken-pox to herpes zoster appeared in 1909, and since then a number of other papers have appeared in which the idea that the two diseases are identical has been supported or opposed. For many years there has been much discussion concerning the interrelationship existing between the various kinds of herpes, and this interest has been stimulated recently by the work of Doerr (12) and others on herpes and lethargic encephalitis. If symptomatic herpes and lethargic encephalitis are caused by the same etiological agent, then the viruses of all the diseases listed in Table I, with the exception of chicken-pox and herpes zoster, have been recovered in some form amenable to experimentation.

Some attempts to transmit varicella to animals and to normal children have already been made. Salmon (6), Tyzzer (13), and Teissier, Gastinel, and Reilly (14) were unable to obtain a specific reaction on rabbits' corneas inoculated with

fresh fluid from varicella vesicles. Swellengrebel (15) found cellular changes in rabbits' corneas inoculated with vesicle lymph, but these changes were not considered characteristic of chicken-pox. Bertarelli (16) and Gins (17), however, reported that, in rabbits' corneas inoculated with fresh vesicle lymph, cellular changes occurred which were specific for varicella and were not present in the controls. Levaditi (18) found Guanieri bodies in rabbits' corneas inoculated either with vaccine virus or with varicella lymph. Park (19), Martin (20), and Tyzzer (13) were unable to transmit chicken-pox to monkeys. Hess and Unger (21) failed to produce varicella in normal children by inoculating them upon the mucous membranes of the nose and throat with vesicle lymph and material collected from the nose and throat of patients with chicken-pox, or by inoculating them intracutaneously, subcutaneously, or intravenously with fresh vesicle lymph.

TABLE I.

An Arrangement of Certain Diseases to Show How Closely They May be Related as Evidenced by Reports of Clinical Observations and of Experimental Work.

	Sheep-pox.
	Horse-pox.
1	Cow-pox.
2	Smallpox.
	Varioloid.
3	Alastrim.
4	Chicken-pox.
5	Herpes zoster.
6	Symptomatic herpes.
	Lethargic encephalitis.

Many workers have attempted to vaccinate normal children against varicella, using fresh vesicle lymph for this purpose. Although most observers[1] have reported only negative results, Steiner (22), Kling (23), Lapidus (24), Meyer (25), Gyr (26), Hotzen (27), and others have reported that the inoculations were followed by positive results in certain cases. These investigators state that after such inoculations some of the children had mild chicken-pox with no local reaction; in other children a local reaction and a mild chicken-pox occurred; in others only a

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[1] See Tyzzer's paper for further references.

local reaction at the site of inoculation appeared; and in still others, often the largest group (26, 27), no reaction of any kind was noticed. All of the writers agree that the vesicle fluid should be collected during the first few days of the eruption and used shortly afterwards. Kling (23) stated that to obtain positive results at least six inoculations should be made, and that even then only one may be successful. These reports seem to indicate that the virus in the vesicles either is very dilute or has lost its virulence.

In view of the conflicting reports by various investigators who used vesicle fluid in their experimental work on the transmission of varicella to man and to animals, it seemed advisable to search elsewhere in the body for the virus of chicken-pox in the hope that thence it might be obtained in a condition more suitable for experimental purposes. Certain observations soon afforded indirect evidence that the virus occurs in the blood and that the amount there does not necessarily correspond to the number of skin lesions. It was noticed that irritation seemed to have a direct influence on the localization of the virus in the skin as evidenced by the appearance of an unusual number of vesicles at the site of irritation. The picture was particularly striking when the irritation involved the skin of the face and extremities, parts of the body usually least affected by the eruption of chicken-pox.

The effect of irritation on the localization of the virus in the skin has been observed in 5 of 51 patients. A brief summary of these observations follows.

B. K. had adhesive plaster applied to the ankles to alleviate the pain of chronic arthritis. After the patient developed varicella the adhesive was removed. Beneath it were found more vesicles than on all the rest of the body (Fig. 1).

B. S and J. M. wore napkins which irritated the skin and the eruption localized mostly in the irritated areas (Figs. 2 and 3).

J. G. wore soft collars with his necktie drawn very tight. Just beneath where the tie rested was a band of vesicles extending around the neck (Fig. 4).

V. S., a young adult, had acne over the face and upper part of the back. The eruption was most marked over these areas and actually involved the acne lesions.

From reports in the literature it was found that the eruption of many of the exanthemata can be made to localize by irritation, provided the irritant is applied before or shortly after the appearance of the eruption. Von Pirquet (28) and Schick (29) have demonstrated this phenomenon in measles, Heim and John (30) in scarlet fever, Swoboda (31) in chicken-pox, and Hebra (10), Tièche (32), and many others in smallpox. Hebra also noticed that in patients with ichthyosis the eruption of smallpox occurred only on the scrotum and on other parts of the body where the skin was fairly normal. Calmette and Guérin (33), Gins (34), Camus (35), and Levaditi and Nicolau (8) have shown that vaccine virus injected intravenously into rabbits can be made to localize in the skin if certain areas be injured immediately after the injection by shaving or pulling out the hair. Camus (35) demonstrated that the localization of the virus under these conditions is not dependent on the presence of injured tissue, but upon the dilatation of the small vessels in the skin.

The observations on patients mentioned above indicate that the virus of chicken-pox probably is present in the blood, and it was thought that it might be possible to demonstrate the disease in animals by injecting them with patients' blood, then shaving and irritating the skin. Before proceeding, however, with the experimental work on varicella, it seemed advisable to determine whether there is a practical method of demonstrating vaccine virus in the blood of animals previously inoculated on the skin. The following experiment, therefore, was performed.

Rabbit A was shaved on both sides of the body. The skin was scarified gently and then inoculated with vaccine virus.[2] A confluent eruption occurred at the site of inoculation. 4 days after the inoculation the skin over the thorax was dissected back,[3] and 10 cc. of blood were removed from the heart. This blood was injected immediately into the ear vein of Rabbit B. A small portion of skin was shaved and scarified shortly after the injection. 5 days later several nodules (Fig. 5) appeared in the shaved skin. These developed into typical vaccine pustules. 2 weeks after the lesions had healed the rabbit was vaccinated on the skin and was found to be immune.

Although the above experiment showed that vaccine virus can be recovered from the blood of animals, it has been impossible to demonstrate in the same way the presence of the virus of varicella in the blood of patients. Other methods, then, were sought. In view of the fact that Pasteur (36) was able to grow the virus of rabies in the brain

[2] The vaccine virus used in this work was supplied by Dr. Noguchi.

[3] All operations were performed under ether anesthesia.

of animals, and that a similar method has recently been employed successfully in the growth of herpes virus, an attempt was made to demonstrate the virus of chicken-pox by injecting small quantities of fresh blood and vesicle fluid from patients directly into the brain of young rabbits. Only negative results were obtained with this method. Then, since the testicles of rabbits have already been shown to be a suitable place for the growth of spirochetes (37, 38), vaccine virus (39), and tumors (40), they were thought of as possibly being a suitable place in which to grow and concentrate the virus of varicella.

Further evidence of the advantage of using the testicles as a place to cultivate a virus was found in the work of Ohtawara (41). He recently reported that he was able to demonstrate vaccine virus in the blood of rabbits vaccinated on the skin by injecting the blood into the testicles of normal rabbits. We have confirmed his work.

Rabbits were vaccinated over extensive areas on both sides of the body. 4 days later the skin over the thorax was dissected back, blood was removed from the heart, and 1 cc. was injected into each testicle of several normal rabbits. After 4 days the testicles were removed, ground up with sand, and tested for the presence of the virus by application of the emulsion to the shaved skin of normal rabbits. Confluent eruptions were obtained with the testicular material, whereas 1 cc. of blood, similar to that injected into the testicles, caused no visible reaction when ground up and smeared directly on the scarified skin.

The demonstration of vaccine virus in the blood of rabbits inoculated on the skin was relatively easy because the virus (39) used in the experiment had been cultivated in the testicles of rabbits for a number of years and can now be transferred without difficulty from rabbit to rabbit by testicular inoculation. According to Dr. Noguchi,[4] however, difficulty was encountered in the original cultivation of the virus in the testicles and it was not until after the fifth or sixth successful passage of the virus that he was certain it could be transferred indefinitely from rabbit to rabbit in this manner. In view of Noguchi's experience with vaccine virus one might expect to encounter trouble in demonstrating the presence of the virus of varicella in the blood of patients by injecting the blood into the testicles of rabbits. It was decided, therefore, in working with this unknown virus to use the well
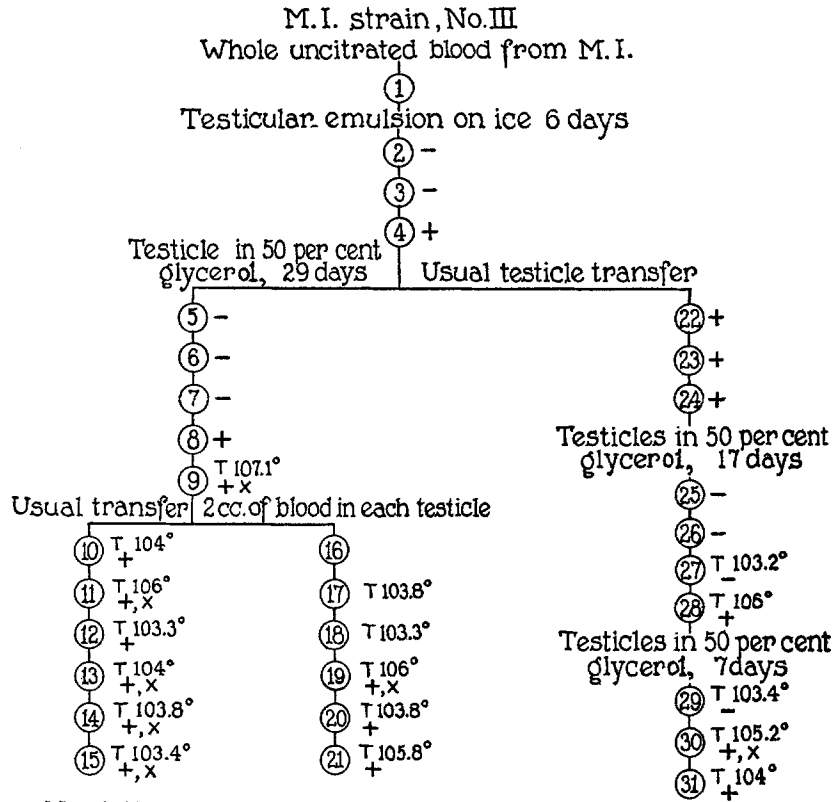
[4] Personal communication.

known laboratory procedure of increasing the virulence of a virus by passing it through a number of animals. The time of transfers is important, for if they are made too early a sufficient growth of the virus to prevent its loss eventually by dilution may not have occurred and if they are made too late the virus may be either dead or damaged to such an extent that it becomes weaker with each passage and finally dies. Plans were made, therefore, to inject blood from a number of patients with varicella into the testicles of rabbits and to make repeated transfers from testicle to testicle at various intervals of time, in the hope that in a few instances the virus would become concentrated enough to manifest itself in the shaved skin. Intervals of 4 days were first chosen for the transfers. This choice was made arbitrarily because the proper time to transfer vaccine virus from rabbit to rabbit is at intervals of 3 to 4 days. In working with the virus of varicella, it proved unnecessary to try other intervals, as every 4 days was soon found to be the correct time for the transfer of this virus also.

The details of the method employed in recovering a virus from the blood of varicella patients are as follows: Blood was drawn from patients with chicken-pox usually during the first 24 hours after the appearance of the eruption. The blood was not citrated and before clotting occurred was injected in 2 cc. amounts into each testicle of normal rabbits (1,800 gm.). These large quantities of blood were used intentionally. At the time of inoculation the needle was moved about in the tissues to produce a certain amount of trauma. 4 days later the testicles were removed, ground up thoroughly with sterile, chemically clean sand, and mixed with 10 cc. of physiological salt solution. The mixture was allowed to stand until the sand settled to the bottom. Strict asepsis was observed throughout the work. Portions of the testicular emulsion were tested for the presence of ordinary bacteria by means of cultures on blood agar, in broth, and in Smith-Noguchi tubes. Other portions for future use were stored on ice either in the original state, or after the addition of equal quantities of glycerol. Then 1 cc. of the emulsion was injected into each testicle of normal rabbits. Two areas on the rabbits' skin were shaved and scarified. One of the areas was smeared with the emulsion, the other was used as a control. An eye of each rabbit was also inoculated.

Both corneas were scarified with a cataract knife (cocaine anesthesia was always used); one was inoculated with the testicular emulsion, the other was used as a control. The first few animals in each series showed little reaction other than that which might be expected to follow the trauma of the inoculations. The skin and cornea healed rapidly. The scrotum was edematous at times for 24 to 48 hours. The testicles, when removed, were slightly swollen. Necrotic areas and often remains of the material injected studded the tissue in various places. This reaction was no more striking than that caused by the injection of an emulsion of normal testicles. The first few rabbits in each series and all the rabbits in the series from which no virus was recovered served as excellent controls for the work. After four to eight transfers of the virus from rabbit to rabbit, however, in certain series, the testicles became tense and firm on the 3rd or 4th day after the inoculation, and the scrotum often remained edematous. In the inoculated eye a roughness of the cornea and a circumcorneal redness appeared on the 3rd or 4th day following the inoculation. An erythema and swelling not present in the control occurred along the lines of scarification in the skin 4 to 6 days after the inoculation (Fig. 6). The testicles, when removed from the animals, were usually swollen and hemorrhagic. Later, when the virus became more concentrated, the inoculated animals looked sick, refused to eat, lost weight, and occasionally developed a diarrhea. At first the temperatures of the rabbits were not elevated. Later, however, it was not unusual for the rabbits to have temperatures of 106–107°F. on the 3rd or 4th day after the inoculation. Temperatures of 104° and 105° occurred frequently (Text-figs. 1 to 5). Occasionally, in addition to the reaction in the skin inoculated with the testicular emulsion, discrete, papular lesions appeared 5 to 11 days after the inoculation in the control areas (Fig. 7). This phenomenon seemed to indicate that the virus invaded the blood stream of the rabbits and became localized in the irritated skin. Invasion of the blood stream was then demonstrated by removing blood from these rabbits and injecting it into the testicles of other rabbits (Text-figs. 1, 2, b, and 5, b).

Blood from eleven patients with varicella was injected into the testicles of rabbits and transplants from each were made through at

least eight rabbits. It was possible in this way to recover a virus from five of the patients. Only the results obtained with the blood

M.I. strain, No.III
Whole uncitrated blood from M.I.
①
Testicular emulsion on ice 6 days
② −
③ −
④ +
Testicle in 50 per cent |
glycerol, 29 days | Usual testicle transfer

⑤ −   ㉒ +
⑥ −   ㉓ +
⑦ −   ㉔ +
⑧ +   Testicles in 50 per cent
⑨ T 107.1°   glycerol, 17 days
    + x   ㉕ −
Usual transfer | 2 cc. of blood in each testicle   ㉖ −
⑩ T 104°   ⑯   ㉗ T 103.2°
     +   ㉘ T 106°
⑪ T 106°   ⑰ T 103.8°   +
    +, x   Testicles in 50 per cent
⑫ T 103.3°   ⑱ T 103.3°   glycerol, 7 days
    +   ㉙ T 103.4°
⑬ T 104°   ⑲ T 106°   −
    +, x   +, x   ㉚ T 105.2°
⑭ T 103.8°   ⑳ T 103.8°   +, x
    +, x   +   ㉛ T 104°
⑮ T 103.4°   ㉑ T 105.8°   +
    +, x   +

− No visible take in skin or cornea
+ Visible take either in eye or skin
× Eruption in shaved areas not inoculated, indicating that virus came by way of blood from testicles
T Temperature

TEXT-FIG. 1. Outline of the method pursued in the study of Virus III. Rabbit 9 was inoculated in the testicles in the usual manner. 5 days after the inoculation, however, the virus was recovered from the blood of this animal and demonstrated by testicular passages through several rabbits.

of M. I., from which Virus III was recovered, will be presented and discussed in detail (Text-fig. 1). It was in the fourth rabbit of the series that a reaction was first noticed, in the eye' skin, and testicles,

more severe than that usually caused by the trauma of inoculation. This reaction became more evident with each passage until the seventh, when a confluent eruption was obtained in the skin of Rabbit 24.
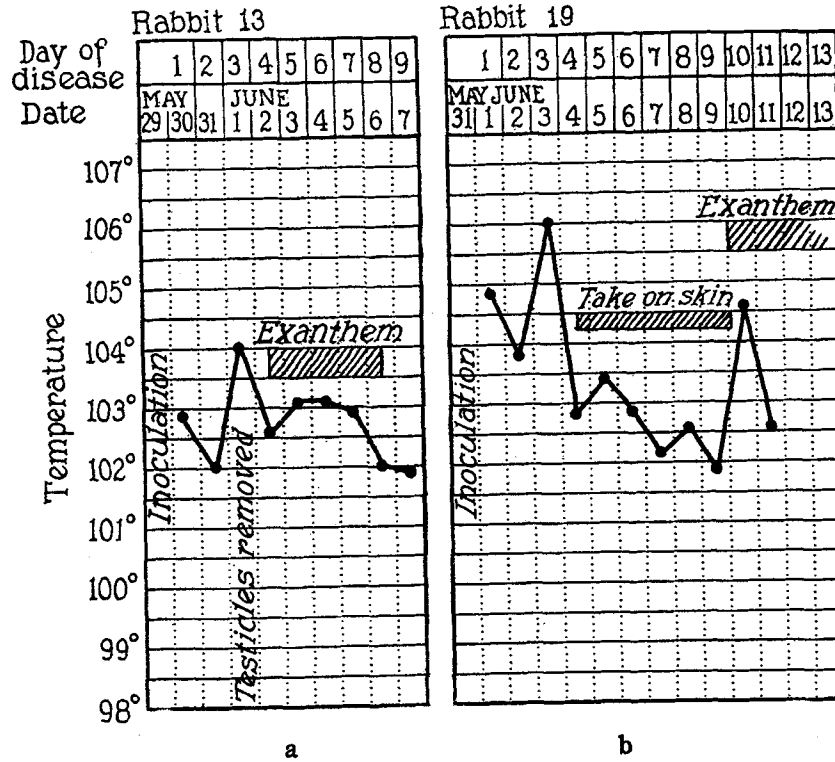
TEXT-FIG. 2, *a* and *b*. (*a*) The rabbit was inoculated in the testicles with Virus III. The chart shows the type of temperature reaction, the time of appearance and the duration of exanthem. (*b*) The rabbit was inoculated in the testicles and on the skin with Virus III which was recovered from the blood of Rabbit 9. The chart shows the type of temperature reaction, the time of appearance and the duration of take on inoculated skin. Coincident with the second rise in temperature, red papules appeared in the uninoculated shaved skin.

The virus was still alive in the testicular emulsion from Rabbit 1, after preservation for 6 days on ice in physiological salt solution as inoculation of it showed (Text-fig. 1). A whole testicle from Rabbit 4 was stored 29 days on ice in 50 per cent glycerol. Then it was

ground up and passed through rabbits in the usual way, and while the virus was still active, its virulence had decreased to such an extent that three testicular passages were necessary before it caused a
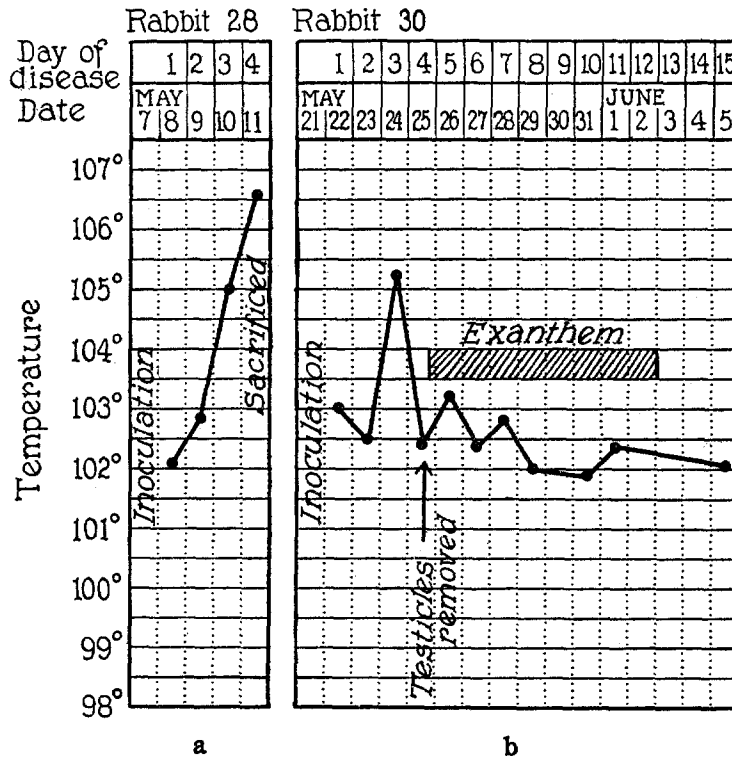
TEXT-FIG. 3, *a* and *b*.   (*a*) The rabbit was inoculated in the testicles and on the skin with Virus III.   The chart shows the type of temperature reaction. The animal was killed and autopsied 4 days after the inoculation.   Nothing except the testicular lesion was found to account for the fever.   (*b*) The rabbit was inoculated in the testicles and on the skin with Virus III.   The chart shows the type of temperature reaction, the time of appearance and the duration of the exanthem which appeared in the uninoculated shaved skin.

visible reaction in the skin.   The virus from Rabbit 24 also showed a decrease in virulence after it had been on ice 17 days in 50 per cent glycerol.   The virus from Rabbit 28, preserved on ice 7 days in glycerol, required only one passage to revive its virulence.   From these

observations it is evident that Virus III survived at least for 6 days in physiological salt solution and for 29 days in 50 per cent glycerol at ice box temperature. Its virulence was decreased, but by a few passages through rabbits this was usually restored.
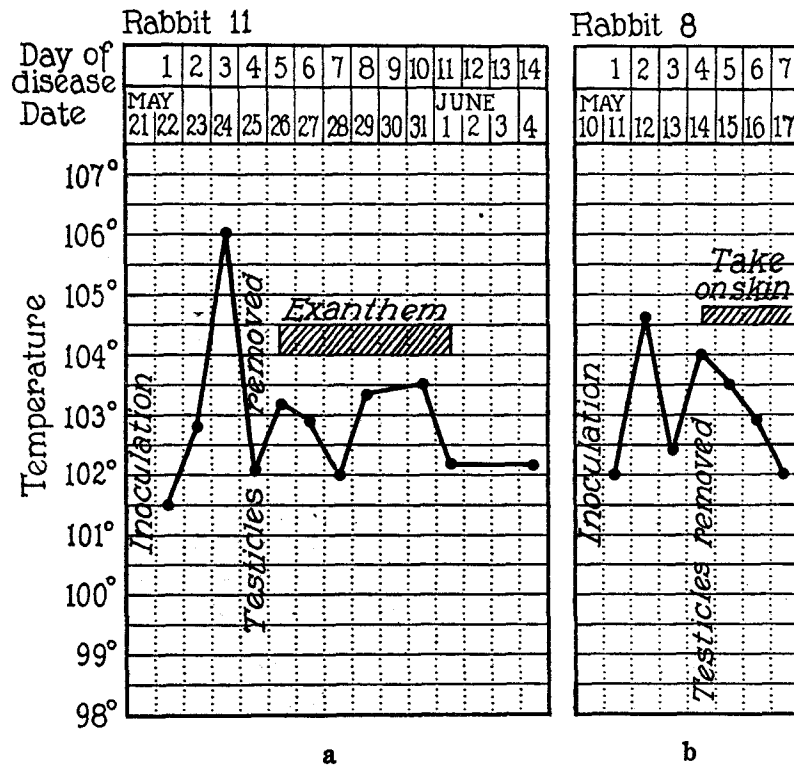
TEXT-FIG. 4, a and b. The rabbits were inoculated in the testicles and on the skin with Virus III. The charts show the type of temperature reaction, the time of appearance and the duration of skin take and exanthem.

Scarified corneas inoculated with Virus III showed a reaction which was not present in the controls. The reaction was not so severe as that caused by vaccine virus or the virus of herpes. It was noticeable in 3 or 4 days, and persisted for 4 to 5 days. The corneas became rough and opaque, especially along the lines of scarification. There were increased lacrimation, photophobia, and injection of

the blood vessels around the cornea. The lesions healed rapidly without permanent injury to the eye unless there was a secondary infection. A reaction in the eye occurred less constantly than in the skin. In sections of the inoculated cornea there was found along
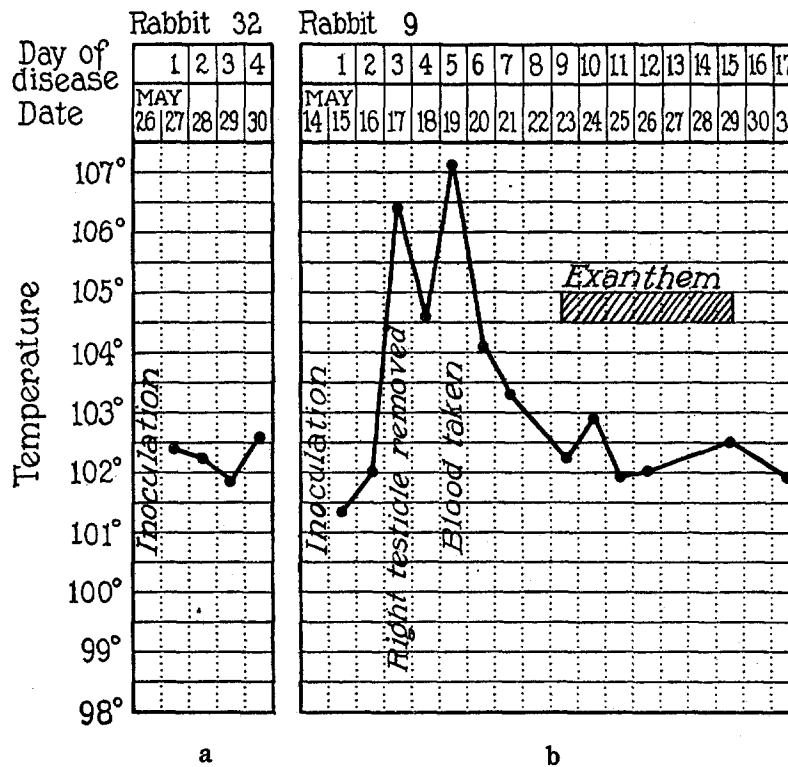
TEXT-FIG. 5, *a* and *b*. (*a*) The usual temperature reaction in animals inoculated with a testicular emulsion free from virus. (*b*) Chart of temperature reaction and time of appearance and duration of exanthem in a rabbit from whose blood the virus was recovered. The animal was inoculated in the testicles with Virus III.

the lines of scarification a type of degeneration involving the nucleus and cytoplasm of the cells similar to the cellular changes found in chicken-pox lesions in the human skin. Occasionally small vesicles were seen in the thick layer of epithelial cells filling the defect in the cornea produced by the scarification.

Discrete lesions, following the inoculation of Virus III on the scarified skin, were small, superficial, red papules which might easily be overlooked. When the virus was concentrated enough to cause confluent lesions along the scarifications, however, the reaction was not so likely to be missed (Fig. 6). The reaction caused by the scarifications in the control skin disappeared by the 3rd or 4th day, while in the inoculated skin the erythema and the swelling of the tissues became more marked at this time, lasted 3 to 6 days longer, and then rapidly disappeared without leaving any scars. Positive results were not always obtained in the skin, even when there were reasons to justify a belief that the virus was present in the material used for the inoculation. Success seemed dependent in part at least upon the condition of the rabbits, the texture of the skin, and the depth of the scarifications. The best results were obtained when the rabbits were healthy and fat, when the skin was rather thick and firm, when the scarifications were deep, but not quite deep enough to cause bleeding, and when the virus was rubbed in thoroughly.

The pathological changes in the testicles were disregarded at first because of the amount of material injected each time. Large quantities were necessary to adapt the virus to the animals. Later, however, when the virus was more concentrated, small quantities of the supernatant fluid from thoroughly centrifuged testicular emulsions were injected. Even after these small injections the testicles would become swollen and tense in 3 to 4 days. When the testicles were removed for examination, or for passage to another animal, they were red, swollen or edematous, with whitish areas scattered over the surface. Upon sectioning them the swollen condition became more evident, as the parenchyma bulged out from the restraining capsule. This reaction is similar to, but less severe than, that caused by vaccine virus.

After the virus became adapted to rabbits, a general reaction was often observed in addition to the local ones in the eye, skin, and testicles inoculated. The rabbits looked sick, refused to eat, lost weight, occasionally developed a diarrhea, and at times had temperatures of 104–107° (Text-figs. 1 to 5). It is true that the temperatures of rabbits vary a great deal, but, as a rule, even in rabbits a temperature above 104° is pathological and one of 105–107° certainly is. The

rabbits did not always have fever, even when marked local reactions occurred in the eye, skin, and testicles. On the other hand, in some of the animals which had high temperatures, discrete lesions appeared in the control areas of the skin or in the shaved skin of rabbits inoculated only in the testicles (Fig. 7). These lesions appeared 5 to 11 days after the inoculation, endured 3 to 7 days, and disappeared without scarring (Text-figs. 1 to 5). They were red macules and papules of various sizes. Sometimes a zone of erythema, less intense than that of the papules, surrounded each lesion. The intensity of the erythema depended a good deal on the color and texture of the rabbit's skin. Coincident with the healing of the lesions, scales formed, and a brownish pigmentation of the skin appeared at these points and persisted for a number of days.

Two types of lesions were observed in the skin of rabbits as the result of shaving alone. One of these was a diffuse or macular erythema, most evident 24 to 48 hours after the shaving, disappearing rapidly and not visible after 4 to 5 days; the other consisted of small whitish nodules of uniform size without redness in or around them, appearing 10 to 14 days after the shaving and usually remaining for a long time. In contrast to the non-specific lesions, certain other lesions occurred in the uninoculated shaved skin of rabbits into the testicles of which Virus III was injected. These lesions, red macules and papules, appeared 5 to 11 days after the inoculation, disappeared rapidly, and were interpreted by us as the result of a general infection with the virus. Whether or not the discrete lesions represent a specific reaction on the part of the skin to a virus carried there by the blood, there can be no doubt that the virus has been recovered from the heart's blood of a rabbit inoculated only in the testicles (Text-figs. 1, 2, b, and 5, b).

Small pieces of skin showing the different types of lesions were removed for histological examination. A description of the pathological changes which occurred in the experimental animals and a comparison with those found in the lesions of human chicken-pox will be made in detail in another paper. In brief, however, the inoculated skin along the lines of scarification showed a cellular infiltration of the corium, a swelling and thickening of the epidermis, and certain intracellular changes similar to those observed in the papular lesions of human chicken-pox.

It proved impossible to predict with regularity whether a visible reaction would occur in any individual rabbit even when a concentrated virus was smeared on the scarified skin. This, to a certain extent, interfered with the demonstration of an immunity in recovered animals and with the determination of the effect of various temperatures on the virus. Work on these points is still in progress and will be reported later. Evidence has been obtained, however, that the virus is destroyed at a temperature of 70°C. for 30 minutes; that at least three of the strains are identical; and that protection against these three strains is not secured by an immunity to vaccine virus.

DISCUSSION.

A great deal has been written about the identity of chicken-pox and herpes zoster. Parounagian and Goodman (42) believe that some of the cases of varicella and herpes zoster reported as occurring simultaneously in the same patient are only instances of generalized herpes zoster. From observations given in the present paper it seems at least possible that certain of the cases may be instances of the localization of the virus of varicella in areas of irritated skin in such a way as to produce zoster-like lesions. Though herpes zoster and chicken-pox may possibly be identical, convincing proof is lacking, in spite of de Lange's (43) work on complement fixation in zoster and varicella. Tièche (32) reported that irritation causes herpetiform lesions in smallpox and modified smallpox, and that such an occurrence is rare in chicken-pox. He emphasized this point in the differential diagnosis of the diseases. One should be careful, however, not to place too much dependence in such a statement, as in 10 per cent of our patients with chicken-pox irritation of the skin caused a localization of the virus in the affected areas.

In working with material supposed to contain a virus which is invisible, or not recognizable, and about which practically nothing is known experimentally, one can easily be mistaken about the identity of the virus or even in regard to its presence. In spite of these difficulties we feel warranted in saying that a virus has been recovered from patients with varicella under the conditions outlined, and that the reactions observed in rabbits are more than the results of a nonspecific irritation. Further evidence must be obtained, however,

before one can think and speak definitely of this virus as the etiological agent of varicella. The ultimate proof depends upon the type of reaction this virus will cause in man and upon the possibility of protecting against varicella by inoculations of the virus.

CONCLUSIONS.

1. The localization of the virus of varicella in the human skin is influenced by irritation. This is indirect evidence that the virus is in the blood.

2. Rabbits are susceptible to a virus recovered from the blood of varicella patients.

3. Testicular emulsions containing the virus are free from ordinary aerobic and anaerobic bacteria, and produce lesions in the cornea, skin, and testicles of rabbits.

4. The virus can be transmitted indefinitely from rabbit to rabbit by means of testicular inoculation, and can be preserved 29 days in 50 per cent glycerol at a low temperature.

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EXPLANATION OF PLATES.

PLATES 38 AND 39.

FIGS. 1 to 4. Photographs showing the effect of irritation on the localization of the virus of varicella in the human skin.

FIG. 5. Two typical vaccine pustules in the skin of a rabbit. The virus was localized in the skin by irritation after the intravenous injection of 10 cc. of blood from a rabbit vaccinated 4 days previously.

PLATE 40.

FIG. 6. (*a*) Rabbit 8. Confluent eruption along the lines of scarifications in the skin inoculated with Virus III. (*b*) Control area in the same animal. 5th day after the inoculation.

FIG. 7. Discrete papular skin lesions in Rabbit 30. The rabbit was inoculated in the testicles with Virus III. The skin was shaved and irritated immediately afterwards. The virus invaded the blood stream and the irritation caused it to localize in the shaved area. The drawing was made on the 6th day after the inoculation.

(Rivers and Tillett: Studies on varicella.)

a. b.
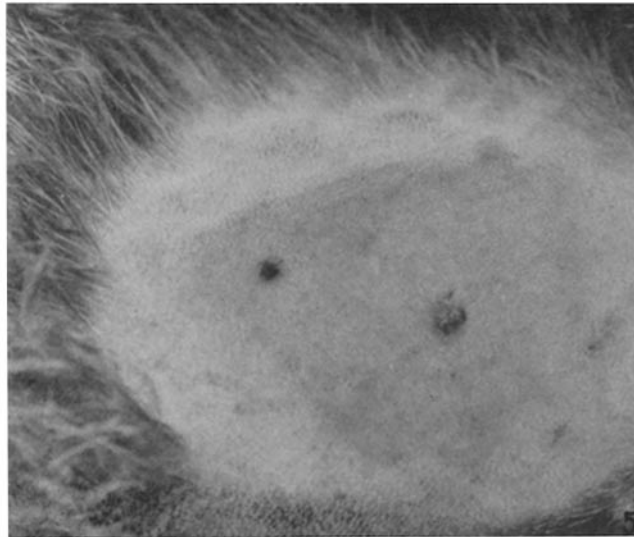
FIG. 6.

FIG. 7.

(Rivers and Tillett: Studies on varicella.)