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# I. A METHOD FOR ESTIMATING THE POTENCY OF SMALLPOX VACCINE

BY

JOHN N. FORCE and JAMES P. LEAKE

# II. THE IMMUNOLOGICAL RELATIONSHIP OF ALASTRIM AND MILD SMALLPOX

BY

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# A METHOD FOR ESTIMATING THE POTENCY OF SMALLPOX VACCINE

By JOHN N. FORCE, Special Expert, and JAMES P. LEAKE, Surgeon, United States Public Health Service

The following communication describes a method for testing the potency of smallpox vaccine which has been developed at the Hygienic Laboratory. It has been held that the potency of smallpox vaccine, in contrast to that of other biologic products, is self-evident in use, and that vaccines of low potency, by the natural process of selection, will be forced off the market. Unfortunately, this has not proved to be the case. Many vaccinations are performed without adequate observation of the results. Physicians have been content to accept a considerable percentage of failures in vaccination. An even more serious difficulty is that, counting on the possibility of relatively low potencies, methods of vaccination have been used such as cross-scarification and the vaccination of large areas which, though tending to give "takes," even with vaccines relatively inert, also tend to cause more slowly healing vaccinia lesions and larger scars, thus discrediting the practice of vaccination.

Various methods for estimating the potency of smallpox vaccine have been developed in this country and in Europe. However, most of the publications on this subject have appeared in European journals.

## I. METHODS INVOLVING THE USE OF THE RABBIT AS THE RECIPIENT OF THE VACCINE TO BE TESTED

1. *Method of Calmette and Guérin.*—Gailleton (1889) and Bard and Leclerc (1891) demonstrated that the rabbit could be employed as a vaccinifer. Lymph from the lesions produced by vaccinating rabbits cutaneously produced typical vaccinia vesicles on children, after passage through calves. The original method suggested by Calmette for testing the potency of smallpox vaccine consisted of the inoculation of incisions made in the ear of the rabbit. At the end of 96 hours, the vaccinated site was examined and the resulting lesions classified according to six degrees of irritation and infiltration. Only vaccines of the sixth degree were considered suitable for release. Later Calmette and Guérin (1901) described a method which they

had employed for the preceding two years in the Pasteur Institute of Lille. They found that samples giving mediocre pustules on calves and children produced no results on rabbits, while samples giving good results on calves and children gave eruptions regularly on rabbits. Since only very potent samples of vaccine gave good eruptions on rabbits, they advised the testing of each lot of lymph by rabbit vaccinations. This procedure assured a margin of safety not possible by using the calf as a test animal.

Calmette and Guérin laid great stress on the manner of applying the smallpox vaccine to the skin of the rabbit, maintaining that scarification involving the derma, or any procedure which would cause the slightest effusion of blood, would result in failure to produce pustules, or at most would result in imperfect pustules, such as appear on a calf inoculated with a deteriorated vaccine. The results following such scarification were extremely variable. The same vaccine would produce results ranging from complete failure to, in rare instances, a typically umbilicated eruption. On the other hand, a confluent eruption was readily produced by simply spreading the vaccine on the freshly shaven skin, without causing any other abrasion of the epidermis than that which would be produced by the irritation of the razor.

An intense congestion of the vaccinated area appeared 48 hours after vaccination, the affected portion being elevated above the surrounding white skin. Vesiculation took place during the third day, the vesicles being sharply umbilicated especially at the edges of the congested area. Some of the vesicles at this time began to show yellowish crusts. The quality of the vaccine was determined from an observation on the fifth day, at which time a collection was made, if desired. The lesions dried rapidly and the crusts separated on the eleventh or twelfth day following vaccination. Immunity to revaccination was present on the sixth day. The details of this technique were as follows:

The backs of three albino or other white-skinned rabbits were shaved from the scapular to the iliac region. The vaccine was diluted with sterile distilled water 1:100, 1:500, and 1:1,000. The dilutions were passed through very fine silk and 1 cubic centimeter of a single dilution was spread on the back of each rabbit. A vaccine of excellent quality produced a confluent eruption with the dilutions 1:100 and 1:500, and at least three or four vesicles per square centimeter of vaccinated skin with the 1:1,000 dilution. If the 1:100 dilution produced no more than three or four vesicles to the square centimeter, the vaccine was rejected.

This technique was later modified by Guérin (1905), who, using the sharp edge of a razor, rubbed 0.5 cubic centimeter of each of the

above three dilutions on the back of each of two rabbits. He also suggested that vaccines should be graded according to the dilutions which would produce three or four vesicles to the square centimeter. These grades were indicated arbitrarily by the numbers 10, 15, and 20. A vaccine graded 10 should not produce more than three or four vesicles per square centimeter with a 1:100 dilution; one graded 15 should not produce more than three or four vesicles per square centimeter with a 1:500 dilution, while one graded 20 should produce at least three or four vesicles per square centimeter with a 1:1,000 dilution. If a vaccine was graded 10 it was considered mediocre. If it did not produce more than three or four vesicles to the square centimeter in a dilution of 1:50 it should be rejected.

This modification has the advantage of eliminating the error due to a difference in receptivity of the three rabbits employed for testing the three dilutions of a single vaccine.

Kelsch (1905) rubbed each of three 50 square centimeter areas, into which the shaven back had previously been divided, with the squarely broken-off tip of a glass pipette filled with one of the three dilutions. On the fourth or fifth day, active vaccines would produce an eruption of umbilicated vesicles the size of a hempseed. The use of pipettes tended to correct the irregularities due to the razor and to produce a more uniform inoculation of the vaccine. Two rabbits inoculated with 1 cubic centimeter of vaccine by this pipette method produced on 180 square centimeters of skin 760 and 620 vesicles, respectively, while two control rabbits inoculated by Guérin's modification of the original method gave 155 and 160 vesicles, respectively. The method of Calmette and Guérin, as modified by Kelsch, is employed by Camus in the Institut Supérieur de Vaccine de l'Académie de Médecine, where the official tests on smallpox vaccine from the producing establishments of France are conducted.

A further modification of the method of Calmette and Guérin was introduced by Belin (1912), working in Chaumier's Institute at Tours. He pointed out that none of the methods so far devised took sufficient account of the variation in receptivity of different rabbits, or of the variation produced by the irritation of the razor in the original method of Calmette and Guérin, or of the pipette in the modification of Kelsch.

He therefore suggested placing the vaccine to be tested on one side of the rabbit and a control vaccine on the other side, in order to reduce the error due to receptivity. He further suggested the use of the two halves of a freshly broken pipette, in order to equalize the amount of vaccine applied to each side of the rabbit and the irritation of its application. Belin developed the following technique, based on these principles:

A rabbit is shaved from the scapular to the iliac region leaving a narrow band of hair along the vertebral column. The two shaved

areas correspond in size. Fresh 1:1,000 dilutions of the vaccine to be tested, and of a control whose potency is known, are made at the same time. A piece of sterile glass tubing is heated, drawn out in the middle, and broken at the center of the constricted portion. One of these pipettes is charged with 0.5 cubic centimeter of the diluted vaccine to be tested and is rubbed with strokes parallel to the vertebral column, working from the vertebræ outward. The vaccine is fed from the pipette at such a rate that drops do not coalesce and form streams running over the field of operation. The friction is continued until the area is congested but not until blood is drawn. The other pipette is charged with the diluted control vaccine and rubbed on the other side of the rabbit, care being taken to cease the irritation when the resulting redness is of the same intensity as on the first side.

Under these conditions the potency of the test vaccine can be compared with that of the control vaccine, (1) because the receptivity of the animal need not be taken into account; (2) because the shaving has been done in exactly the same manner on the two sides; (3) because, even when there was a slight difference in the irritation produced by the shaving, the difference disappeared after friction with the pipette which results in an irritation absolutely identical on both sides.

In support of his contention that this method eliminates the source of error in the method of Calmette and Guérin, Belin presents a photograph of a rabbit showing a vaccine which would have been considered of low potency, had it not been observed that the control vaccine of known high potency gave an even less satisfactory eruption on the same rabbit. Differences in receptivity of various regions of the same rabbit do not influence the result because comparsions between the test virus and its control are made by symmetrical regions. Belin asserts that differences of potency can be detected by this method which would pass unnoticed among primary vaccinias of children.

Groth (1921), using the method of Calmette and Guérin, had difficulty in segregating the eruptions due to the various dilutions even when adhesive tape was used to separate skin areas. He believed that 0.5 cubic centimeter was too large an amount of dilution to apply to each of three areas on one animal, and used only 0.2 cubic centimeter of a 1:100 dilution. With a rubber-covered finger, this dilution was rubbed into the freshly scraped back of a white rabbit which had been depilated with calcium hydrosulphide six to eight days previously. After four or five days the number of isolated pustules were counted and 10 pustules were added for each square centimeter of confluent eruption. This total multiplied by 500 represented approximately the number of organisms in 1 cubic centimeter of undiluted vaccine. A range from 67,500 to 290,000 organisms per

cubic centimeter was found in 25 vaccines tested by this method. In the test of certain of these vaccines on children, Groth was disappointed to find that the results were fairly constant—i. e., the diameter of the pustule, its degree of development, and the intensity of inflammation of the surrounding area did not vary in proportion to the number of organisms per cubic centimeter of vaccine. In his article, Groth mentions the work of Belin, and it is remarkable that he was not sufficiently impressed by Belin's arguments to use a control of known high potency in carrying out this quantitative determination. Had he done this, and rejected any vaccine with low count, when the control count was low on the same animal, it is probable that the agreement between his human and his rabbit results would have been greater. The assignment of an arbitrary value of 10 to each square centimeter of confluent eruption is open to objection, since it is manifestly impossible to compute the number of organisms giving rise to a confluent eruption.

Groth, however, was able to secure agreement between rabbit and human results by the subepidermal injection of 0.1 cubic centimeter of each of five dilutions of the vaccine ranging from 1:10 to 1:100,000. The local reactions arising from these injections were arbitrarily classified from 5 to 1 according to intensity of duration and redness, and their diameters were measured at the end of the third or during the fourth day. The sum of the five intensity classifications (maximum, 25) was designated the "potency index," and the sum of the five diameters was designated the "infiltration diameter." Groth states that a good vaccine should have a potency index over 18 and an infiltration diameter over 50. In a series of vaccines tested he found that the potency index ranged from 9 to 25 and the infiltration diameter ranged from 21.1 to 72 millimeters. While these ranges were greater than the ranges found in testing the same vaccines on children, Groth was able to show a general agreement. For example, a group of vaccines with an average pustule diameter under 5.1 millimeters in children gave an average infiltration diameter of 45.9 millimeters in rabbits, and a similar group with an average pustule diameter over 6 millimeters gave an average infiltration diameter of 54 millimeters.

Recently one of the authors (J. N. F.) working with Beattie compared the subepidermal method of Groth with the cutaneous method described in this article (pp. 12–16), while testing the potency of 1:100 dilutions of smallpox vaccine which has been exposed to room temperature for one, two, and three weeks. It was found that the subepidermal method using these 1:100 dilutions gave no result other than a traumatic redness which faded at the end of 24 hours, while 1:1,000 dilutions, made from the same 1:100 dilutions, by the cutaneous method gave six, five, and one isolated vesicles for the dilutions which had been kept at room temperature for one, two, and three weeks,

respectively. Control dilutions which had been kept in cold storage for the same periods, when tested by the method of Groth, gave infiltrations measuring 15, 16, and 19 millimeters, respectively, and, when tested by the Hygienic Laboratory method, gave 100 per cent, 98 per cent, and 100 per cent confluent eruptions.

It appears, therefore, that the Hygienic Laboratory method permitted the detection of residual living organisms in a 1:1,000 dilution of a vaccine, while a 1:100 dilution of this same vaccine produced no result when injected subepidermally.

Gordon (1925) apparently has not encountered the difficulty with the scarification method which was pointed out by Calmette and Guérin. He divides the skin of the rabbit into a number of areas and places 0.02 cubic centimeter of the dilution to be tested in each area. The skin is then lightly cross scratched through the drop of vaccine. A vesicle occurring on the fifth day is considered evidence of a positive result. The dilutions used are 1:100, 1:1,000, 1:10,000, and 1:100,000. The potency is expressed in terms of minimal vaccinating doses (m. v. d.) in each milligram of the vaccine. For example, if a vaccine is positive in 1:10,000 and negative in 1:100,000 the 20 milligrams applied to the area represent 10,000 m. v. d. or 500 m. v. d. per milligram.

Henseval and Convent (1912) have devised a modification of the method of Calmette and Guérin based on the neutralizing power of the serum of rabbits previously immunized against vaccinia.

The entire back of a rabbit is inoculated with a 1:50 dilution of a potent vaccine in order to secure an abundant confluent eruption. Eighteen days later the rabbit is bled, the serum separated, placed in vials (1 or 2 cubic centimeters to each vial), and dried in vacuum. At the time of use the serum volume is restored by adding distilled water. The vaccine to be tested is diluted 1:250, three 0.5 cubic centimeter portions of this dilution are mixed with 0.2, 0.05, and 0.01 cubic centimeter, respectively, of the immune serum, and the volume of each mixture brought to 1 cubic centimeter with physiological salt solution. The mixtures represent 1 volume of vaccine to 100, 25, and 5 volumes of serum, respectively. After an hour at 37° C., 0.5 cubic centimeter of each of the three mixtures is inoculated on 60 square centimeters of the shaven back of one rabbit. The resulting eruption is observed on the fifth day and the vaccine classified as follows:

| 1 volume of vaccine mixed with— | Good | Fair | Poor |
|---|---|---|---|
| 100 volumes of serum | Complete neutralization. | Complete neutralization. | Complete neutralization. |
| 25 volumes of serum | Incomplete neutralization. | do | Do. |
| 1 volume of serum | 150 lesions | 100 lesions | Do |

Fig. 1.—Illustrating the use of stencil plate in laying off equal skin areas for test. This test was not in the regular series for bulk vaccine as finally adopted (dilutions 1:1000, 1:3000, 1:10,000 and 1:30,000) but was a modification of Henseval's test for potency, using varying dilutions of a vaccinia-immune serum mixed with constant amounts of diluted vaccine. The modification consisted principally in the use of symmetrical control areas (Fig. 2) inoculated with mixtures of vaccine and normal serum. The ratio of vaccine to serum on the four areas is 1:100, 1:30, 1:10 and 1:3
The virucidal effect of the higher concentration of serum is well shown. Rabbit 61

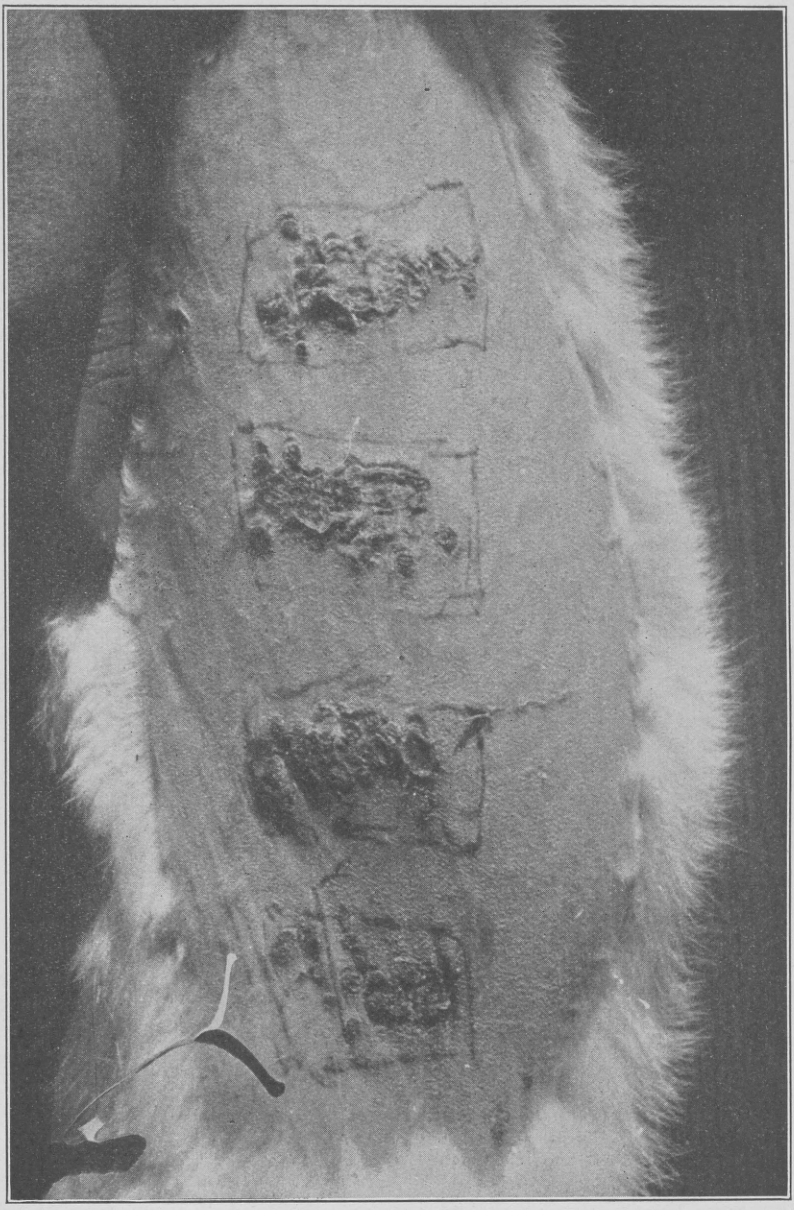

Fig. 2.—Control to Fig. 1, right side of same rabbit, showing that normal rabbit serum, in dilutions of 1 : 100 to 1 : 3⅓ has little or no virucidal property. Rabbit 61

One hundred and fifty lesions on 60 square centimeters of skin is considered a rich eruption, while an eruption of 12 lesions on an area of the same size is considered complete neutralization. An eruption consisting of less than 100 lesions but more than 12 is considered incomplete neutralization.

The authors of this method do not advocate it as a substitute for that of Calmette and Guérin or for that of Chaumier, but recommend it as a method based on a different principle, to be applied as a check on the other two.

Henseval and Convent do not mention the use of nonimmune rabbit serum in order to control possible inherent neutralizing action, or the use of more than one rabbit in the test in order to control receptivity. These additions might enhance the value of the test. In a trial of the neutralization method, accordingly, the technique of Henseval and Convent was modified by introducing the use of a control animal, as follows:

A vaccine known to give 75 per cent to 100 per cent confluent eruptions in a dilution of 1:3,000 was diluted 1:1,500 and mixed with equal amounts of 1:15, 1:50, 1:150, and 1:500 dilutions of normal rabbit serum and of serum from a rabbit which had recovered from vaccinia. The resulting mixtures represented a 1:3,000 dilution of vaccine combined with 1:30, 1:100, 1:300, and 1:1,000 dilutions of serum. In terms of volume, one volume of vaccine was mixed with 100, 30, 10, and 3 volumes of serum, respectively. The four mixtures and the four controls were inoculated, in the manner hereinafter described, on the backs of two rabbits, the highest concentration of serum being inoculated on the area farthest anterior. Table I presents the results of this test in terms of eruptions observed at the end of one week. (Figs. 1 and 2.)

TABLE I.—*Neutralization test of smallpox vaccine*

| 1 volume of vaccine mixed with— | Rabbit 61 | | Rabbit 62 | |
|---|---|---|---|---|
| | Normal serum | Immune serum | Normal serum | Immune serum |
| 100 volumes of serum | 95 per cent confluent. | 3 lesions | 10 lesions | 1 lesion. |
| 30 volumes of serum | do | 50 per cent confluent. | 99 per cent confluent. | 8 lesions. |
| 10 volumes of serum | 100 per cent confluent. | 40 per cent confluent. | 95 per cent confluent. | 80 per cent confluent. |
| 3 volumes of serum | 95 per cent confluent. | 75 per cent confluent. | 99 per cent confluent. | 95 per cent confluent. |

In accordance with the standards suggested by Henseval and Convent this would be considered an excellent vaccine, since it was not completely neutralized by less than 100 volumes of immune serum on the first rabbit. The paucity of the eruption on the first

area with normal serum in rabbit 62 shows the occasional low receptivity which may cloud results unless controls are used.

2. *Method of Gorini.*—The various methods involving the application of smallpox vaccine to the depilated or shaved back of the rabbit having been reviewed, for the sake of completeness, mention is made of the method of Gorini. Gorini (1903) inoculates the corneas of rabbits with the vaccine to be tested and examines them microscopically at the end of three days. The test is purely qualitative, no quantitative results being possible. In an article on the control of smallpox vaccine published in the monthly bulletin of the Office International d'Hygiène Publique (1912) this method is condemned on the ground that, in addition to requiring great care in technique, it is too sensitive, so that vaccines of low potency are overrated.

The general opinion concerning the use of the rabbit as the recipient of tests for the potency of smallpox vaccine is well expressed in the following sentence from the same bulletin: "It is thus seen that the results of the method of testing vaccine in the rabbit depend in a large measure on the operative procedure. They are always sufficiently constant for practical needs. The essential point is to follow a predetermined procedure and to know what will result with vaccines of known potency."

NOTE—Since this manuscript was submitted for publication, a meeting has been held at Berlin, January 13, 1927, of the smallpox and vaccination commission of the Health Committee of the League of Nations under the chairmanship of Professor Ricardo Jorge, director general of the Public Health Service, Portugal. The modification of the rabbit method in use at the Hygienic Laboratory and described in this paper, pages 12–16, was not before the commission, which decided that a vaccine may be considered potent if, when ready for use and applied to an experimental animal in the dilution of 1 to 1,000 with physiological saline without filtration, it will produce the following results at the end of three days:

1. *Method of Calmette-Guérin.*—1 cubic centimeter applied over the entire shaven back of a rabbit should produce three to four vesicles to the square centimeter. (See pp. 1–3.)

2. *Method of Gins.*—The dilution rubbed into the scarified cornea of a guinea pig should produce marked opacity of the entire cornea. (See Gorini above.)

3. *Method of Groth.*—0.1 cubic centimeter injected into the depilated skin of the back of a rabbit should produce a clearly visible and palpable area of local infiltration and redness. (See pp. 5 and 6.)

4. *Method of Sobernheim.*—0.9 cubic centimeter rubbed into three incisions, each 4 centimeters long, on the depilated skin of the back of a rabbit should give redness and isolated papules, or a papular infiltration, along the lines of incision.

## II. METHOD INVOLVING THE USE OF THE CALF AS THE RECIPIENT OF THE VACCINE TO BE TESTED

The calf has been used as a recipient for potency tests for many years, following a procedure first advocated by Warlomont (1883). The French vaccination law prescribes that a place shall be reserved

on all calves being vaccinated at the Institut Supérieur in Paris for the control of the potency of other vaccines.

A single calf is used to test several samples of vaccine. These are inoculated by parallel incisions on regions of the skin of different degrees of susceptibility, accompanied in each instance by a control vaccine of known potency. The use of several regions guards against accidental failure in one region, and the use of a vaccine of known potency controls the difference in susceptibility of different regions of the skin. The potency of the vaccine is determined from a consideration of the type, development, and quantity of vesicles obtained.

Cunningham and Cruickshank (1923) have introduced a commendable modification in the calf test by the use of dilutions, estimating the number of discrete vesicles per linear inch for each dilution. Each two lots are tested in duplicate on two calves, the entire shaven surface of each calf being used for the 50 linear insertions of the different dilutions. No control vaccine was used by them.

The testing of vaccine samples on a calf at the time of inoculation for vaccine production has been criticized on the ground that no potency results can be accepted as positive from tests made in a part of the establishment where routine work with vaccines is in progress, or from tests made on an animal inoculated with a virus propagated on the same species of animals and therefore adapted to that species.

## III. METHOD INVOLVING THE USE OF THE GUINEA PIG AS THE RECIPIENT OF THE VACCINE TO BE TESTED

For fully twenty-five years, the vaccine producers in this country have been testing the potency of vaccine by rubbing it into linear scratches made on the scrotum of a guinea pig. The advantages of the use of these animals are that one or more separate animals can be used for each vaccine to be tested and that the tests need not be carried on where the vaccine is produced. The sole disadvantage arises from the small size of the receptive area, which barely suffices for a single insertion and its control, and which eliminates all possibility of the use of dilutions of a test vaccine and its control on one animal. (See note, above, p. 8, method of Gins.)

## IV. METHOD INVOLVING THE USE OF THE CHILD AS THE RECIPIENT OF THE VACCINE TO BE TESTED

*Method of Chaumier.*—Chaumier (1910) inoculates the vaccine to be tested into several scratches 1 centimeter in length on one arm of a previously unvaccinated child and a vaccine of known potency on the other arm. At the end of 72 hours potent vaccine should show a long vesicle with a regular or slightly indented border. Vaccines

of poor quality show a row of isolated circular vesicles. The observation must not be delayed beyond the three-day period, for the isolated vesicles will unite later and present an appearance indistinguishable from that produced by a good vaccine. Chaumier recognizes eight degrees of result, ranging from complete vesiculation to complete failure (fig. 3, upper row).

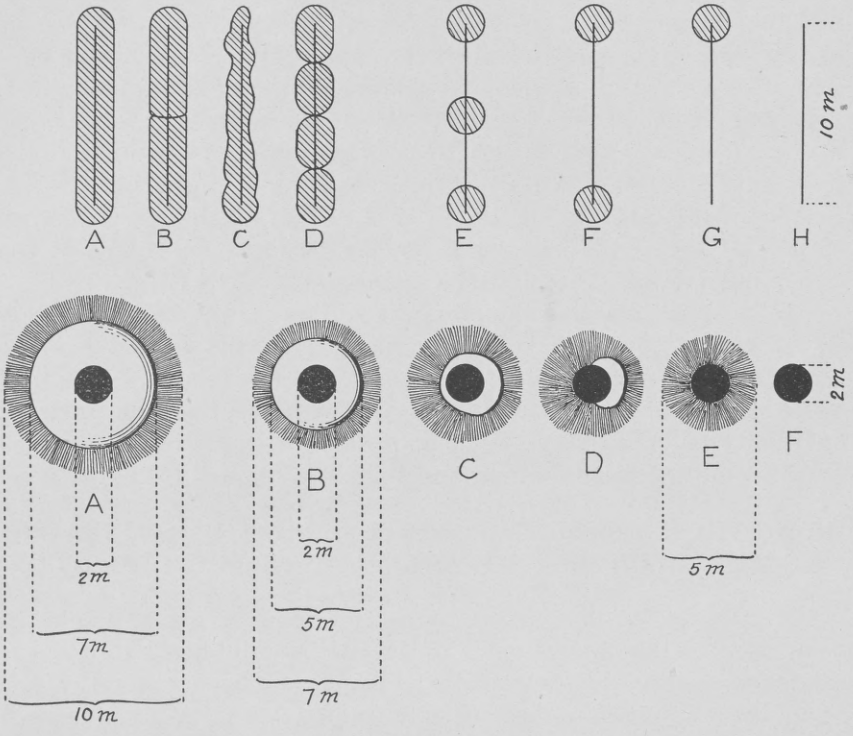


FIG. 3. Diagrammatic illustration of the lesions of primary human vaccinia as a guide for the estimation of the potency of different vaccines. (Magnified 2.3 diameters)

Method of Chaumier, insertion by scratch 10 mm. long. Observation 72 hours after vaccination:

A. Excellent (vesiculation complete and regular).
B. Good (vesiculation nearly complete).
C. Good (vesiculation irregular).
D. Fair (vesiculation broken, but contiguous).
E. Poor (isolated vesicles).
F. Very poor (two vesicles).
G. Practically inert (one vesicle).
H. Complete failure (no vesiculation).

Method of authors, insertion by drill 2 mm. wide. Central black circle indicates area of drill scarification. Observation 7 days after vaccination:

A. Good, full vesiculation, at least 7 mm. in diameter.
B. Fair, full vesiculation, at least 5 mm. in diameter.
C. Poor, irregular vesiculation less than 5 mm. diameter.
D. Very poor crescentic vesiculation.
E. Almost inert, papule and areola only (not to be confused with the early reaction of immunity in previously vaccinated subjects).
F. Complete failure, crust only, from trauma of drill.

Chaumier states that in France "the majority vaccinate by means of pricks and introduce only a very minute quantity of fluid; if, on a scratch 1 centimeter in length, covered with vaccine, only a single point has shown a vesicle, one understands how many chances there are for the vaccine inserted by means of pricks not to produce any result."

Groth (1921) uses the method of Chaumier, but has different criteria for estimating potency. He inserts the vaccine to be tested into 4 scratches on 1 arm of each of 10 previously unvaccinated children and observes them at the end of 7 days. The resulting pustules are graded as 3, 2, or 1, according to the amount of development, a grade of 2 representing the ordinary pustule. A mean of the 40 pustule grades is determined which is designated the "pustule potency index." In like manner, if the areas of redness around the 4 pustules are confluent, area formation is graded 3; if area formation has begun a grade of 2 is assigned, while a grade of 1 indicates that the vesicles are surrounded only by the narrow red areola. The mean of the 40 area grades is designated the "area potency index." Vaccines with pustule and area values below 1.5 are considered below standard. A third criterion known as the "pustule diameter" is established by measuring the 40 pustules in the narrow diameter with calipers calibrated to 0.1 millimeter and computing the mean of these measurements. Vaccines producing pustules with diameters below 4.5 millimeters are considered below standard.

If previously unvaccinated children are not available, the vaccine may be tested on 50 children, vaccinated approximately 10 years previously. Resulting pustules are graded 3, vesicles 2, and papules 1, the grades are added and divided by the number of subjects. The result is designated the "revaccination index," its maximum value being 12, i. e., four pustules on each subject. Vaccines giving a revaccination index below 6 are considered below standard.

From the illustrations accompanying Groth's article, it is apparent that the vesicles rated 2 are vaccinoid lesions, and that there is no distinction in grading between the seventh-day papules produced by a vaccine of insufficient potency in a nonimmune subject, and the seventh-day vestiges of papules indicating an immediate reaction of immunity following the vaccination of an immune subject.

Groth makes three adverse criticisms of his own method. First, the insufficiency of suitable human material; second, the absence of a control vaccination by a vaccine of known potency; and third, the length of time required for testing a given vaccine, since it is imperative that a bacteriological test be made of the vaccine before it is employed for the test vaccination of children.

The objections to the use of human subjects for potency tests do not apply to the use of animals, especially rabbits. The objection has been raised that the use, in potency tests, of a species of animal in general less susceptible than man, might result in the rejection of many lots of vaccine capable of giving successful results on human subjects. On the other hand, a vaccine giving good results on animals less susceptible than man has a margin of safety in its expectancy of potency that will help to protect it against the attenuation arising from adverse conditions of temperature during transportation and storage; moreover, the greater danger from the standpoint of smallpox is in the vaccine which is too weak rather than in one which is too strong.

## V. A SUGGESTED MODIFICATION OF THE CALMETTE-GUÉRIN TEST

After consideration of the arguments in support of the various tests, a method has been developed of determining the potency of smallpox vaccine, based on Belin's modification of the method of Calmette and Guérin, which involved the inoculation of rabbits with controlled dilutions of the vaccine to be tested. The results obtained from the inoculation of rabbits with each vaccine tested were compared with the results of vaccination of human subjects showing no vaccination scars. The details of the technique will now be considered:

1. *Choice of rabbits.*—After several disappointing experiences arising from excessive growth of hair and deposition of pigment in gray and New Zealand rabbits it was determined to use only albino rabbits. Even in these animals there are occasional patches of coarse hair. When these patches are shaved, there are exposed areas of coarse skin with large hair follicles. The hair grows out on these patches with remarkable rapidity, tending to obscure the observation of any eruption which may be present. If these patches could not be avoided in laying out the areas for inoculation, the animal was discarded. Groth believes that these "urticaria-like" patches are found principally in rabbits under 8 months old, though he has noted them occasionally in fully matured animals. The chief difficulty experienced with young animals, aside from their lack of size and hardiness, is the rapidly growing fine hair.

2. *Shaving the rabbit.*—Following the suggestions of Calmette and others, the rabbits were shaved immediately preceding the application of the vaccine. It was soon found, however, that a certain amount of fine hair, when moistened, escaped observation and therefore the rabbits were shaved on the day before the test. The hair of the rabbit was clipped with shears having a 5-inch blade, the skin

being protected by slipping a coarse-toothed comb through the portion of hair about to be severed. The clipped area extended from the scapular to the iliac region and about 12 centimeters from the median line down each side, with the exception of a strip 3 centimeters wide along the vertebral column. An abundant lather was then made with a neutral soap, or shaving cream, and warm water, and thoroughly rubbed into the clipped area with the ends of the fingers. The regions were then shaved, keeping the skin well stretched and shaving in the direction of hair growth. Particular attention was paid to keeping the razor sharp in order to avoid cuts and abrasions of the rabbit's skin which would later interfere with the inoculation. After shaving, the skin was repeatedly rinsed, while rubbing with the hand, until all feeling of soapiness had disappeared. The animal was dried with paper towels and returned to its cage.[1]

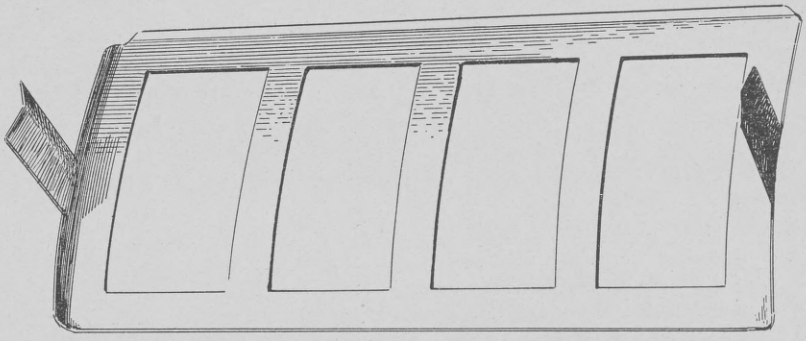

FIG. 4.—Stencil plate used for outlining equal areas on rabbit's back for vaccination. The apertures measure 2.5 by 5 cm.

On the following day, just before applying the vaccine, the rabbit was shaved again, no soap and as little water as possible being used. This resulted in a mild hyperæmia of the skin, similar to that which had formerly been produced by means of sandpaper (No. 0) immediately before inoculating the vaccine. It was possible, therefore, to abandon the use of sandpaper.

3. *Marking the rectangles for inoculation.*—At first the shaved area of each side was divided into four divisions by means of three lines drawn with a blue wax pencil from the vertebral column downward. (Figs. 5–9, p. 16.) In order to outline the areas more sharply, separate them from each other and from the adjacent hair, and make them all of one size, a stencil plate (fig. 4) was made from thin galvanized iron. The apertures in the plate measured 2.5 by 5 centimeters. When in use the stencil plate was pressed against the side of

[1] Surg. Charles Armstrong has obtained satisfactory results by anesthetizing the rabbit and plucking the hair from the back instead of shaving. He finds that the removal of the hair from the follicle retards the new growth of hair.

the rabbit by an assistant, while the operator, with a fine-pointed blue wax pencil, traced the outlines of the openings on the shaven skin, which was allowed to remain at its natural tension during the whole process. It was later found that the outlines could be dotted in with a moistened indelible pencil or India ink without moving the skin beneath the stencil plate. By the use of this plate four rectangles were outlined which were of equal size when the rabbit's skin was not stretched. (Figs. 1 and 2, p. 6.)

4. *Making the dilutions of vaccine.*—The dilutions were made in small, sterile, conical glasses previously filled with the amounts of physiological salt solution indicated in the accompanying table. (Table II.) The salt solution was run into the glasses from a burette, marked to correspond to the required amounts.

The control vaccine was kept in storage at 4° C. as a dilution of 1:8 of pulp in 50 per cent glycerinated, distilled water, this being approximately half the concentration of the smallpox vaccine of commerce. A dilution of 1:25 of this half-strength control vaccine would, therefore, correspond to a 1:50 dilution of a commercial vaccine.

TABLE II.—*Method of making serial dilutions*

Commercial vaccine, 0.1 c. c., plus salt solution 9.9 c. c. equals 1 in 100 dilution.

1 c. c. of 1:100 plus 9 c. c. salt solution equals 1:1,000 ..... (A)
1 c. c. of A plus 2 c. c. salt solution equals 1:3,000 ..... (B)
1 c. c. of A plus 9 c. c. salt solution equals 1:10,000 ..... (C)
1 c. c. of B plus 9 c. c. salt solution equals 1:30,000 ..... (D)

Control vaccine (half strength) 0.1 c. c., plus salt solution 2.4 corresponds to 1:50 dilution of a full-strength vaccine.

0.5 c. c. of 1:50 plus 9.5 c. c. salt solution equals 1:1,000 ..... (*a*)
1 c. c. of *a* plus 2 c. c. salt solution equals 1:3,000 ..... (*b*)
1 c. c. of *a* plus 9 c. c. salt solution equals 1:10,000 ..... (*c*)
1 c. c. of *b* plus 9 c. c. salt solution equals 1:30,000 ..... (*d*)

Each pipette used in making these dilutions was filled and emptied 10 times with the tip pressed against the bottom of the glass before transferring liquid from one container to the next. Dilutions were not filtered, as it was desired to retain all active elements in the sample employed.

The above-described method of dilution applies to vaccine received in vials. If the vaccine to be tested was received in capillary tubes, the following procedure was used:

Ten cubic centimeters of sterile salt solution was placed in a conical glass; a capillary tube was wiped off with alcohol, dried with gauze, fitted with a rubber bulb, the tips broken with sterile gauze, and the contents expelled into the glass containing the 10 cubic centimeters of salt solution, the capillary tube being rinsed without allowing the

diluent to enter the bulb. Since the capillary tube was assumed to contan 0.01 cubic centimeter of vaccine, this represented a dilution of 1:1,000. A similar dilution was made from three additional tubes, and four rectangles on a rabbit were inoculated as hereinafter described. This method furnished a check to the uniformity of potency in tubes of the same lot.

5. *Inoculation of the rabbit.*—The first inoculations were made with tapered pipettes, as suggested by Belin. It was found, however, that such pipettes tended to lacerate the skin and to become clogged with tiny particles of epidermis. The even spread of the dilutions was consequently interfered with. Finally pipettes were used as follows: Glass tubing measuring 3 millimeters inside diameter and 5 millimeters outside diameter was cut into 20-centimeter lengths, plugged with cotton, and sterilized. After the dilutions of vaccine had been prepared, one of these pieces of tubing was completely encircled with a file scratch at the center and broken *squarely* off; tubes breaking irregularly were discarded. The two halves were then laid side by side and marked with a file at a level which would indicate approximately 0.4 cubic centimeter capacity. The pieces were then passed through the flame, separated, and laid to cool across the top of the two conical glasses holding the corresponding dilutions of the vaccine to be tested and the control. At first one pair of pipettes was used for applying all the dilutions, but later a pair was used for each dilution, since it was found that even these pipettes eventually became clogged with epidermal scurf.

At the moment of inoculation each dilution was drawn up and emptied out of its pipette 10 times. The pipette was then filled to the scratch and held perpendicularly against the tightly drawn skin of the rectangle to be inoculated. As the pipette was rubbed from front to rear with parallel strokes, the first strokes being made in the dorsal region and each successive stroke farther down toward the flank, the contents were gradually released, care being taken to avoid coalescence of large drops which would spread outside the area. The friction was continued until an even redness, without trace of blood, covered the entire rectangle. The four dilutions were applied on one side; the weakest first, in the posterior rectangle. The control was invariably applied to the right side, the vaccine to be tested to the left side. The process was repeated on a second rabbit, a new set of pipettes being used on this animal. As soon as the inoculation was finished, each rabbit was put in a separate cage in which food had been placed. The rabbit finding food at hand, and not being wet, was not tempted to lick the inoculated sites.

6. *Recording of results.*—At the end of seven days the amount of eruption in each rectangle was recorded in triplicate, one filing card

bearing the number of the rabbit, a second the number of the vaccine being tested, and a third that of the control vaccine, the rectangles being designated *a*, *b*, *c*, *d* on the control side and A, B, C, D on the test side. Results were expressed (*a*) in percentage of the rectangle covered by confluent eruption, or (*b*) by the number of discrete lesions counted, or (*c*) by a combination of the two. For example, 75 per cent 16 would indicate that 75 per cent of one rectangle was covered by a confluent eruption and that there were 16 discrete lesions on the portion not covered by the confluent eruption.

7. *Use of control vaccine.*—The use of a control vaccine did not imply that such vaccine was superior to the vaccine tested, but only that its behavior was known from observation of a series of animals of varying receptivity. (Figs. 5, 6, 7, 8, and 9.) The value of the control is especially well shown (Table IV) in comparing the results of the tests of vaccine S on rabbits 31 and 32, vaccine T on rabbits 51 and 52, and vaccine V on rabbits 33 and 34, vaccine Z on rabbits 39 and 40, and vaccine AA on rabbits 41 and 42. For example the control vaccine on rabbit 31 gave 100 per cent, 95 per cent, 95 per cent, and 50 per cent, while the same control on rabbit 32 gave 21, 19, 5, and 1 discrete lesions in the same dilutions. If the vaccine accompanying this control had been tested by the original method of Calmette and Guérin on rabbit 32, it would have been incorrectly graded as a vaccine of low potency.

The seed virus (New York City Department of Health) from which the control vaccine was grown was collected from a calf inoculated with crusts from human vaccinia. The seed virus was diluted to represent 1:50 of pulp and rubbed with the edge of a scalpel into the freshly shaven backs of albino rabbits. Collection was made on the fifth day, the pulp weighed, diluted 1:8 with 50 per cent glycerinated water, ground by hand in a mortar and stored at 4° C. Each of three rabbit vaccines prepared in this manner was tested by inoculating two spots on a calf, good collections being made from the resulting eruptions.

## VI. VACCINATION OF HUMAN SUBJECTS TO CONTROL THE POTENCY TEST ON RABBITS

The first series of rabbit and human tests was made with capillary tubes from stock commercial packages. (Table III.) The second series of rabbit and human tests was made with vaccines shipped in bulk by the producers who at the same time were asked to send 100 empty capillary tubes. These bulk samples had not been passed for commercial distribution, and it is obvious from the results of the tests herein described that some were below satisfactory potency. (Table IV.) In preparation for the human tests in this series, a

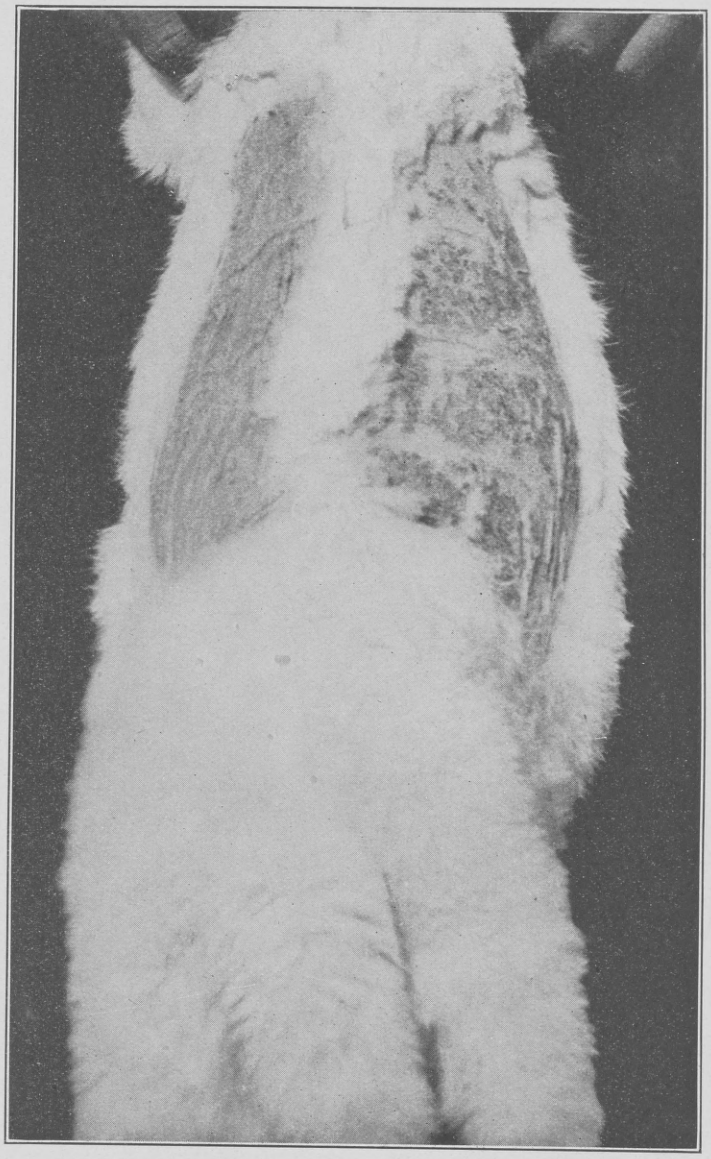

Fig. 5.—Test of tubed vaccine showing the failure of all four tubes tested (left side) as contrasted with confluent eruption produced on corresponding areas with the same dilutions of the control vaccine (right side). Rabbit 5
Note the unshaved strip along the vertebral column separating the test areas from the control

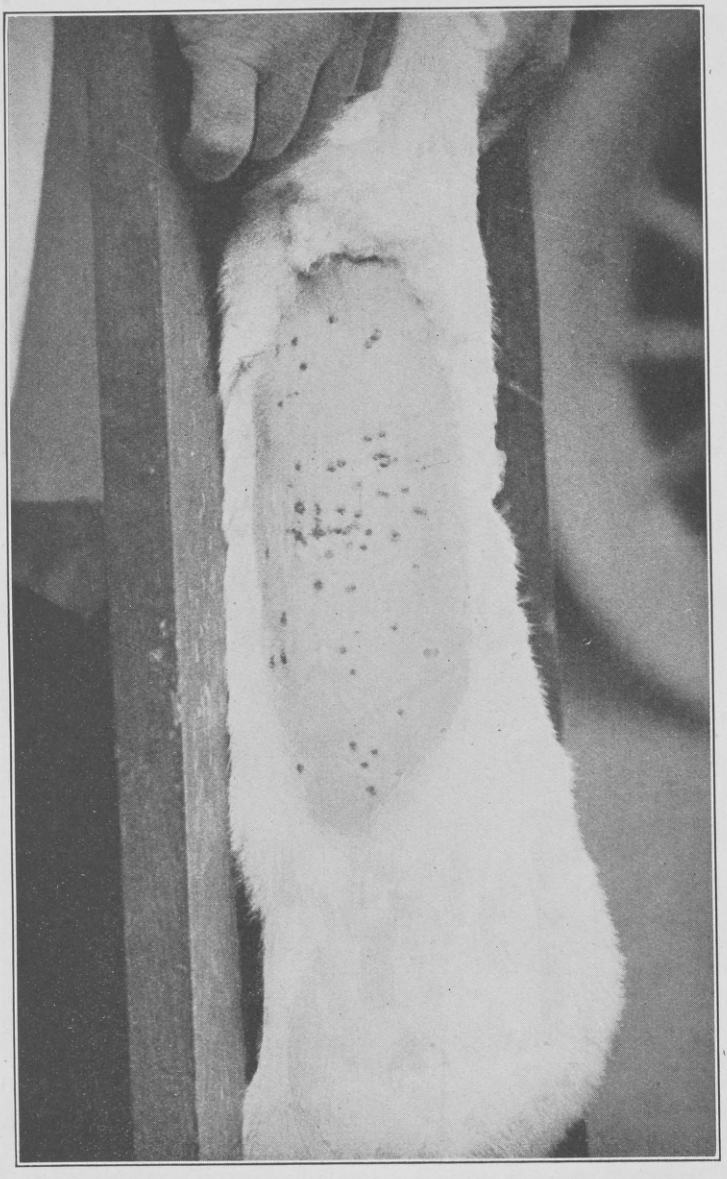

Fig. 6.—Test of tubed vaccine, illustrating the necessity of using a control vaccine for comparison. The four tubes produced a very scant eruption, but this was evidently due to the lack of receptivity on the part of this particular rabbit, as may be seen in Fig. 7. Rabbit 16

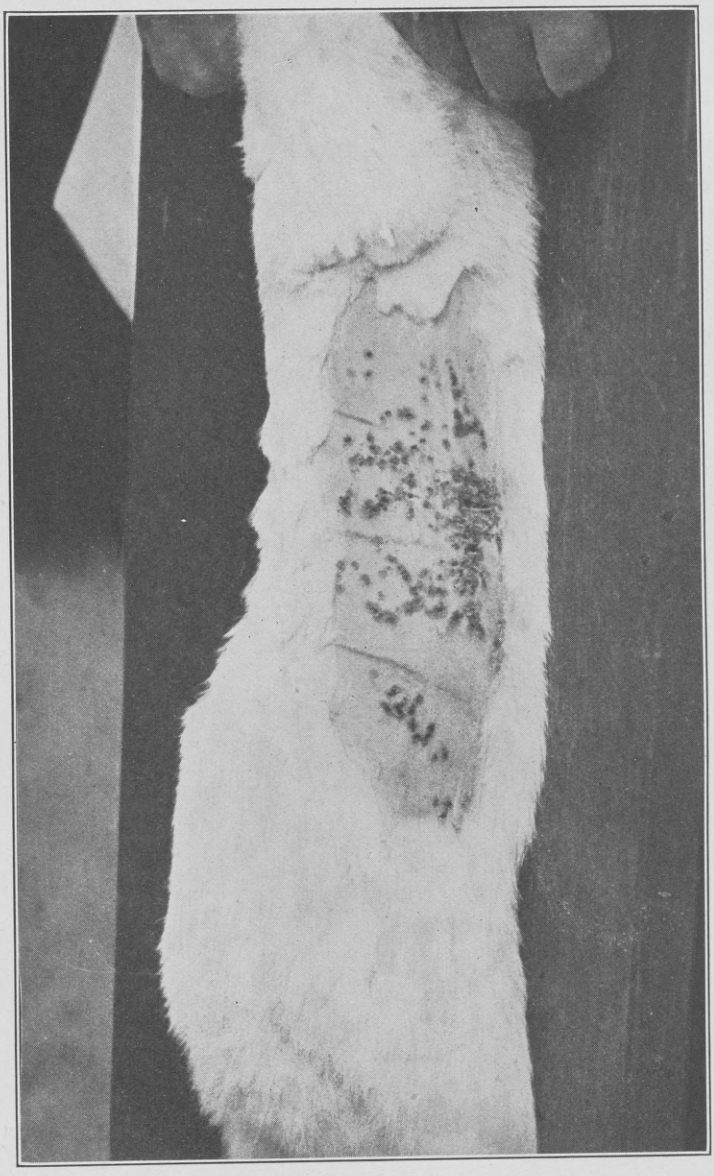

Fig. 7.—Control areas (right side) of rabbit whose test areas are illustrated in Fig. 6. Eruption largely discrete, but failure of confluence is not due to lack of potency of vaccine. (Compare Fig. 9, showing eruption produced by same control vaccine 9 days later). Rabbit 16

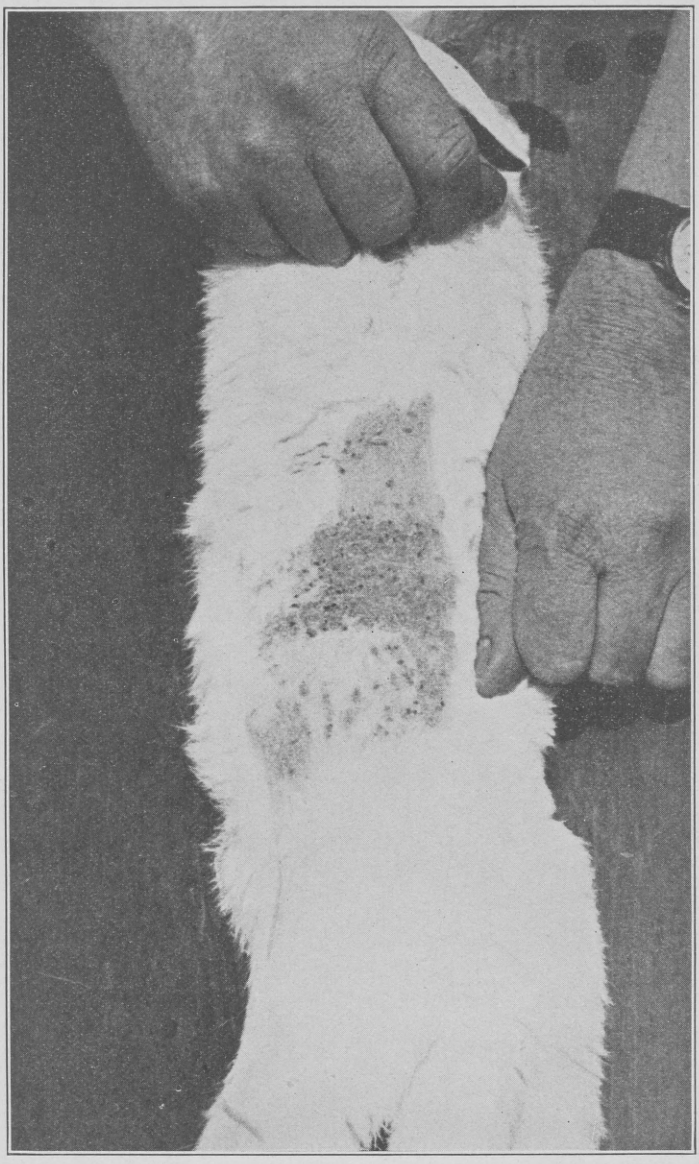

Fig. 8.—Test of tubed vaccine illustrating differences in potency in individual tubes of the same lot. Rabbit 24

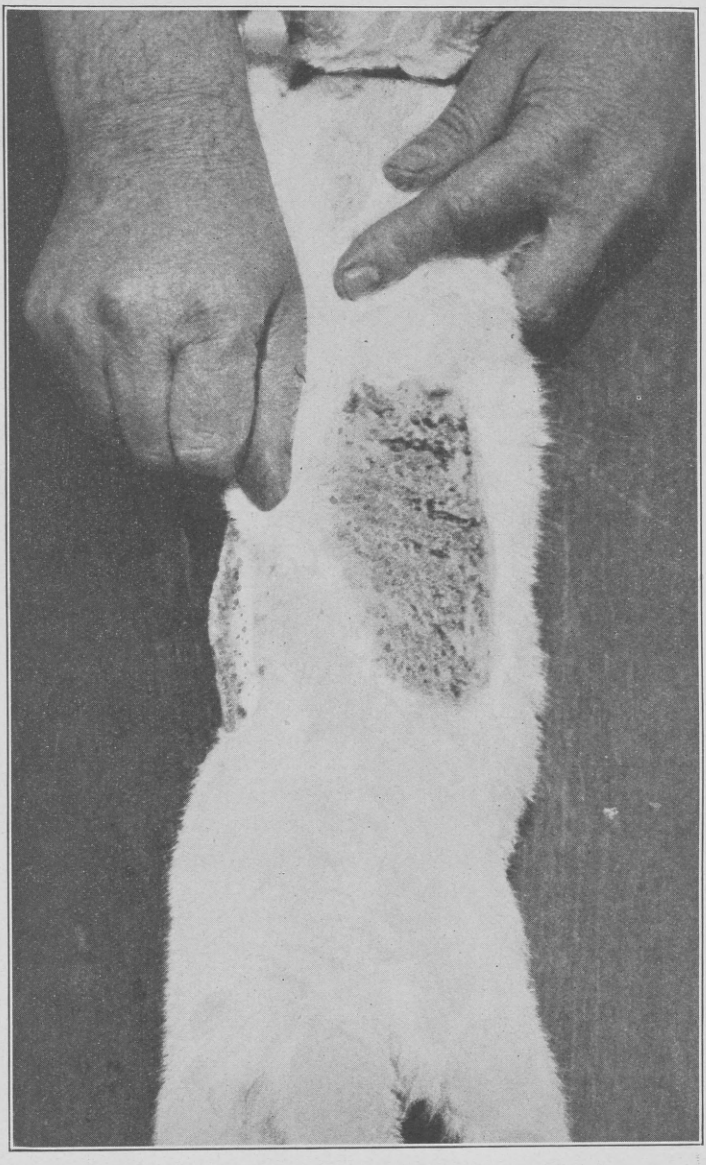

Fig. 9.—Control side (right) of rabbit whose test areas are shown in Fig. 8. The maintenance of potency in the control vaccine is shown by the confluent eruption in this photograph as compared with the areas inoculated with the same control vaccine 9 days previously (Fig. 7) and 37 days previously (Fig. 5, right side)

The dilutions for the tests on tubed vaccine including the control, are all 1:1,000. If varying dilutions are used, as for tests on bulk vaccine, the need for some method of separating the areas is apparent. This need was met by the use of a stencil plate (Fig. 4 and Figs. 1 and 2). Rabbit 24

portion of each vaccine received was transferred to a small vial and another portion was used in filling the accompanying capillary tubes. The vial was returned to cold storage, the tubes left for from two to seven days at room temperature and then placed in an electric refrigerator at approximately −3° C. For each human vaccination of three insertions the contents of two capillary tubes, and that amount from the small vial which would adhere to the blade of a toothpick, were used to inoculate the three scarifications. In general, the percentage of insertion success was in favor of the bulk vaccine in vials which gave, in this series, 82 per cent "good" results as compared with 75 per cent of "good" results from the tubed vaccine.

The vaccines tested on children were taken to the clinic either packed in shaved ice in a vacuum bottle, or, if in original packages, they were carried in an ice-cream freezer also packed with ice. The vaccinations were performed according to the modification of Pirquet's technique suggested by Force (1914), which was selected because the uniform scarification made possible a quantitative expression of results. This consists essentially in applying the vaccine to

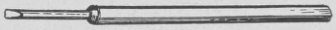

FIG. 10.—Drill used for human vaccination. The portion set into the hexagonal handle is made of carbon steel and will not lose its temper through sterilization by flaming. The drilling edge is square cornered, 2 mm. in width, and sharp

a 2-millimeter circle of derma exposed by removing the epidermis by means of the rotary motion of a small drill (fig. 10) held perpendicularly to the tightly drawn skin. Three scarifications were generally made. The vaccine was rubbed in with the blade of a sterile toothpick for each insertion. When bulk vaccine was used and this rubbing was carried on for 15 seconds, the percentage of "good" results was 90, as against 77 when the vaccine was rubbed in for less than 15 seconds—i. e., simply dabbed in with a few strokes. When tubed vaccine was used and the rubbing was carried on for a period definitely greater than 15 seconds, the percentage of "good" results was 94, as against 74 when the rubbing was continued for exactly 15 seconds, and 71 when the vaccine was rubbed in for less than 15 seconds. The arm was cleaned with acetone or alcohol, and dried before scarification. It was found that the percentage of "good" results was 78 with acetone and 70 with alcohol. The scarification was not made through the drop, as suggested by Pirquet (1907). No dressings were employed.

The vaccinia vesicle produced by this technique is practically circular and is remarkably constant in diameter for any given vaccine. A vesicle measuring 5 millimeters in diameter on the fifth day will

increase at the rate of approximately 1 millimeter daily until it reaches its maximum diameter of approximately 10 millimeters. The majority of such vesicles should attain a diameter of 7 to 8 millimeters at the end of one week after vaccination.

The second series of tests on children was brought to a close by the lack of suitable subjects. When it was apparent that no further tests were possible at that time, the tubed vaccines were placed in cold storage, at 4° C., until a new supply of subjects could be secured.

After a period of approximately two months in cold storage, test tubes containing from 10 to 50 capillary tubes of each of nine of the above lots of vaccine were shipped from Washington, D. C., to Berkeley, Calif. The test tubes of vaccine, together with a test tube containing a thermometer, were placed in the inner compartment of an "auto-vacuum" ice-cream freezer the outer compartment of which had been filled previously with cracked ice. A circle of heavy asbestos board was cut to fit the inside bottom of a small garbage can. The freezer was then packed in sawdust in the garbage can, care being taken that it was centrally placed in relation to the top, bottom, and circumference of the can. The nine lots of vaccine were packed in two cans. A third can containing four lots of vaccine was shipped 20 days later. Both shipments were made during the month of August, the cans being approximately six days in transit.

On arriving in Berkeley, the cans were opened, the thermometers read, and the cans immediately placed in commercial cold storage (4° C.). The temperature of the room where the cans were opened was 25° C., approximately 4° higher than the temperature inside the cans. The two cans comprising the first shipment were removed from the cold-storage plant to the vaccination clinic at the end of one hour, not more than 30 minutes being consumed in transit and in removing the test tubes from the cans and packing them in cracked ice. The tubes comprising the second shipment were removed from the can and placed in cold storage for 125 days, after which they were transferred to the vaccination clinic in an iced container.

Only one lot of vaccine was tested at a time, the capillary tubes being transferred from the test tube to a covered Petri dish resting on cracked ice. The contents of one capillary tube were used for each of two insertions on one subject, a third insertion being made from a vial of high-potency vaccine. The same high-potency vaccine was used in the tests made 125 days later, a fresh vial being removed from cold storage for this purpose.

The results of the tests of 12 of the 13 lots of tubed vaccine mailed from Washington to Berkeley are shown in Table IV. Lot Q was not tested because no more subjects were available.

The high-potency vaccine used for control for the Berkeley tests gave 116 "good" and five "poor" insertion results but no failures when used with the nine lots of the first shipment. A vial of the same vaccine, removed from cold storage for use with the three lots of the second shipment 125 days later, gave 32 "good" and 2 "poor" insertion results and 20 failures. This represents a deterioration from 96 per cent of "good" results to 59 per cent. When a high-potency vaccine suffered so great a deterioration during a period of approximately four months in a commercial cold-storage plant said to be kept at 4° C., the failure of the three lots of the second shipment, after a similar period of storage following a transcontinental trip, is not remarkable.

The subjects of both series of tests were students entering the University of California, without evidence of previous smallpox or without previous vaccination scars. In addition to the above results there were five vaccinoids (four in previously vaccinated subjects showing no scars) and one immediate reaction of immunity, in a previously vaccinated subject showing no scar, observed at the control insertion sites of the first group. Two vaccinoids in previously vaccinated subjects showing no scars were similarly observed in the second group.

In all tests on human subjects a vesicle measuring at least 7 millimeters on the seventh day was graded A, and one measuring at least 5 millimeters on the seventh day was graded B. (Fig. 3, lower row.) Both A and B grades of vesicles were tentatively classified as "good" in recording the results in the accompanying tables. In order to determine the reliability of this standard, 111 seventh-day measurements were taken of vesicles arising from the insertion of the above-mentioned high-potency control in the first group of subjects and 29 similar measurements of the second group.

The following statistical results were obtained:

| Group | Number of vesicles | Mean, in millimeters | Standard deviation, in millimeters | Coefficient of variation |
|---|---|---|---|---|
| I | 111 | 7.6±0.04 | 0.7±0.04 | 9.2±0.42 |
| II | 29 | 6.2± .08 | .6± .05 | 9.7± .87 |

From these figures it is apparent that a standard which admits as "good" all vesicles measuring no less than 5 millimeters on the seventh day is extremely liberal, since the mean of the second group, less one standard deviation, gives a measurement of 5.6 millimeters. However, the control vaccine used in the second group gave only 59 per cent of "good" insertion results. A more satisfactory standard

would be the grade A previously described—i. e., a seventh-day vesicle measuring at least 7 millimeters in diameter—when the scarification has been made with a drill measuring 2 millimeters in width. This agrees closely with the mean pustule diameter of 4.5 millimeters following scratch insertion, suggested as a standard by Groth.

Another point of interest is the extremely small standard deviation in both groups, which indicates a remarkable consistency in the results produced by this method of vaccination.

## VII. TABULATION AND DISCUSSION OF RESULTS

From the records made on the three sets of cards (rabbit, vaccine, and control vaccine) two tables were constructed, summarizing the results of two series of tests, one made on capillary tubes mailed to the laboratory without any attempt at securing a low temperature in transit (Table III), the other made with vaccines shipped in small vials under conditions of temperature which are described in the table (Table IV).

Certain points in these tables are of especial interest. For example, four tubes of vaccine B gave on first test 1 lesion, 1 lesion, 15 per cent confluence, and 9 lesions, respectively. On second test, two weeks later, four tubes of this same vaccine gave 16 lesions, 90 per cent confluence, 70 per cent confluence, and 47 lesions, respectively, although the expiration date set by the producer had been passed 11 days previously. Between these two tests 16 tubes had been used on children with 75 per cent "good" insertion results. This indicates clearly that the margin of safety is on the side of the rabbit test, for there would be no hesitation in condemning a vaccine giving good results on rabbits in only two tubes out of eight tested. In like manner, vaccine C gave fair results in only 1 tube out of 8, but when 17 tubes were tested on children 47 per cent "good" insertion results were obtained.

All eight tubes of vaccine J gave poor results on rabbits, with 50 per cent "good" insertion results on children, while all eight tubes of vaccine I gave poor to mediocre results on rabbits, with 76 per cent "good" insertion results on children. However, it should be recalled that the designation "good," as applied to the results on children, included not only grade A vesicles but grade B as well, and it has already been pointed out that grade B is probably too liberal a standard for inclusion in the classification "good."

The rabbits marked with an asterisk in Table IV had all been used for testing material from persons suspected of having smallpox. In

some instances no eruption had been obtained on these rabbits, but it is evident that varying degrees of immunity had been produced.

The difference between the results obtained from the 1:1,000 and 1:3,000 dilutions is remarkably small when the vaccines are of sufficient potency to produce patches of confluent eruption in both these dilutions. The average difference in this series was only 13 per cent. A vaccine of high potency should produce from 90 to 100 per cent confluence in a dilution of 1:1,000 and not more than a 20 per cent reduction in this confluence in a dilution of 1:3,000. The results with the 1:10,000 and 1:30,000 dilutions serve as checks on the results with the lower dilutions and also assist in differentiating the higher degrees of potency which are necessary before virus is tubed or shipped.

In order better to interpret Table IV, the history of vaccine P is here presented in detail. This vaccine, age unknown, was received at the laboratory in a vacuum bottle, packed in a chest alongside a can of ice, its temperature being 11° C. It was placed in cold storage at 4° C. for 37 days and then tested on rabbits 57 and 58 with excellent results, showing 80 and 75 per cent confluence in a dilution of 1:3,000. Twenty-seven days later the vaccine was taken from cold storage, tubed, and left at room temperature for six days to represent ordinary mailing conditions across the continent. The tubes were then returned to cold storage and held for 52 days, after which they were mailed across the continent in an iced container, reaching their destination in six days with a temperature 4° C. below that of the room where opened. The tubes were then placed in cracked ice and 84 of them were used for vaccination of college entrants showing no vaccination scars. The time from arrival in the laboratory to this final test was 128 days, 6 of which had been spent by the vaccine at room temperature. The final test gave 36 per cent "good" insertion results.

Vaccine M illustrates the agreement between the results of rabbit and of human tests. This vaccine gave excellent results in a dilution of 1:3,000 on rabbits, 100 per cent "good" insertion results in bulk tests on children, and 84 per cent "good" insertion results in tube tests. At the other end of the scale is vaccine Y, with only a few scattered lesions on rabbit tests and no "good" results in either bulk or tube tests on children, and only one "good" out of 28 insertions on college entrants.

Finally, it appears that the combined use of controls and of dilutions on rabbits permits a quantitative determination of the degree of potency of a smallpox vaccine, which parallels rather closely a classification of lesions produced in human subjects, based on the diameter and appearance of seventh-day vesicles.

## VIII. SUMMARY

1. The literature on potency tests for smallpox vaccine is reviewed.

2. A method for making potency tests based on Belin's modification of the method of Calmette and Guérin is described.

3. Tables showing the results of tests of smallpox vaccine on rabbits by this method, and on human subjects by vaccination, using a standard technique, are presented.

4. The agreement between the results of tests on rabbits and on human subjects is discussed.

5. The following criterion of potency is suggested: A smallpox vaccine of high potency, when diluted 1:1,000 should produce a confluent eruption on from 90 per cent to 100 per cent of the vaccinated area on the back of a rabbit, and when diluted 1:3,000 the decrease in confluence should not be over 20 per cent. A vaccine satisfying this criterion should produce, in all previously unvaccinated human subjects, a circular vesicle measuring at least 7 millimeters in diameter on the seventh day when applied, undiluted, to a circle of the exposed derma measuring 2 millimeters in diameter.

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TABLE III.—*Potency test of commercial capillary tubes of smallpox vaccine sent by mail to the Hygienic Laboratory*

| Vaccine | Days in cold storage (4° C.) before test | Days in electric refrigerator (−3° C.) before rabbit test | Days from rabbit test to expiration date of vaccine (minus sign used when expiration date preceded test) | Days control vaccine in cold storage, dilution 1 in 8, before rabbit test | Days control vaccine in electric refrigerator, dilution 1 in 50, before rabbit test | Rabbit No. | Results of vaccination of rabbits with commercial vaccine and control in dilutions of 1 in 1,000 of the contents of 4 capillary tubes, expressed as percentage of area of skin covered by confluent eruption and additional isolated lesions. The control results are placed below those for the commercial vaccine | | | | | | | | Days in electric refrigerator before test on children with no vaccination scars | Days from test on children to expiration date of vaccine (minus sign used when expiration date preceded test) | Results of vaccination of children with tubed vaccine. Figures indicate numbers of insertions; letters indicate classes of lesions (fig. 3, lower row) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Tube 1 | | Tube 2 | | Tube 3 | | Tube 4 | | | | Good A B | Poor C D E | Failure F |
| | | | | | | | % | | % | | % | | % | | | | | | |
| A | 105 | 35 | −97 | 7 | 0 | 8 | 0 | 2 | 0 | 2 | 0 | 2 | 0 | 4 | 63 | −125 | | | 2 |
| | | | | | | | 100 | | 100 | | 50 | | 100 | | | | | | |
| | 105 | 64 | −126 | 29 | 7 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | |
| | | | | | | | 0 | 40 | 0 | 66 | 0 | 41 | 0 | 37 | | | | | |
| B | 34 | 50 | 5 | 15 | 7 | 15 | 0 | 1 | 0 | 1 | 15 | | 0 | 9 | 54 to 61 | 1 to −6 | 12 | 1 | 3 |
| | | | | | | | 0 | 15 | 0 | 35 | 75 | | 75 | | | | | | |
| | 34 | 66 | −11 | 29 | 9 | 24 | 0 | 16 | 90 | | 70 | | 0 | 47 | | | | | |
| | | | | | | | 70 | | 100 | | 80 | | 100 | | | | | | |
| C | 21 | 45 | 16 | 15 | 2 | 11 | 0 | 1 | 0 | 0 | 10 | | 0 | 1 | 54 to 56 | 7 to 5 | 8 | 2 | 7 |
| | | | | | | | 50 | | 75 | | 75 | | 25 | | | | | | |
| | 21 | 66 | −5 | 29 | 9 | 23 | 0 | 19 | 0 | 10 | 30 | | 0 | 11 | | | | | |
| | | | | | | | 95 | | 99 | | 90 | | 95 | | | | | | |
| D | 5 | 49 | 15 | 15 | 6 | 14 | 0 | 7 | 0 | 17 | 0 | 4 | 0 | 2 | 54 to 56 | 10 to 8 | 2 | 5 | 8 |
| | | | | | | | 0 | 20 | 0 | 15 | 30 | | 0 | 32 | | | | | |
| | 5 | 65 | −1 | 29 | 8 | 22 | 0 | 18 | 0 | 20 | 0 | 15 | 0 | 4 | | | | | |
| | | | | | | | 80 | | 95 | | 95 | | 20 | | | | | | |
| E | 21 | 57 | −23 | 29 | 0 | 17 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 61 | −27 | | | 6 |
| | | | | | | | 60 | | 60 | | 85 | | 0 | 3 | | | | | |
| F | 16 | 43 | 6 | 15 | 0 | 9 | 0 | 0 | 0 | 6 | 0 | 2 | 0 | 1 | 47 to 61 | 2 to −12 | 4 | 1 | 14 |
| | | | | | | | 50 | | 50 | | 50 | | 50 | | | | | | |
| | 16 | 64 | −15 | 29 | 7 | 19 | 0 | 7 | 0 | 1 | 0 | 3 | 0 | 7 | | | | | |
| | | | | | | | 0 | 25 | 0 | 18 | 97 | | 70 | | | | | | |
| G | 40 | 57 | −43 | 29 | 0 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 40 | −26 | | | 8 |
| | | | | | | | 0 | 14 | 0 | 5 | 60 | | 40 | | | | | | |
| H | 10 | 13 | 32 | 2 | 0 | 5 | 0 | 0 | 0 | 1 | 0 | 3 | 0 | 3 | 17 to 23 | 15 to 9 | | | 4 |
| | | | | | | | 100 | | 100 | | 100 | | 100 | | | | | | |
| I | 25 | 30 | 30 | 29 | 0 | 16 | 0 | 7 | 0 | 37 | 0 | 10 | 0 | 6 | 34 to 63 | 26 to −3 | 16 | 1 | 4 |
| | | | | | | | 10 | 18 | 60 | | 30 | | 0 | 22 | | | | | |
| | 25 | 66 | −6 | 29 | 9 | 25 | 0 | 49 | 0 | 47 | 0 | 18 | 0 | 19 | | | | | |
| | | | | | | | 70 | | 80 | | 60 | | 60 | | | | | | |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| J | 4 | 43 | 12 | 15 | 0 | 10 | 0 | 1 | 0 | 14 | 0 | 13 | 0 | 1 | 47 | 8 | 6 | 6 |
| | | | | | | | 100 | | 100 | | 100 | | 100 | | | | | |
| | 4 | 65 | −10 | 29 | 8 | 21 | 0 | 0 | 0 | 2 | 0 | 7 | 0 | 4 | | | | |
| | | | | | | | 0 | 29 | 50 | | 0 | 35 | 0 | 59 | | | | |
| K | 29 | 49 | −1 | 15 | 6 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 47 | 1 | | 12 |
| | | | | | | | 100 | | 100 | | 100 | | 100 | | | | | |
| L | 23 | 35 | 22 | 7 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 7 | 34 to 40 | 23 to 17 | | 12 |
| | | | | | | | 100 | | 100 | | 100 | | 100 | | | | | |

TABLE IV.—*Potency test of commercial*

| Vaccine | Container received | Temperature, centigrade, when received | Days in cold storage (4°C.) before test. Days in electric refrigerator (−3°C.) indicated by E. R. | Days control vaccine in cold storage, dilution 1 in 8 before rabbit test | Days control vaccine in electric refrigerator, dilution 1 in 50, before rabbit test | Rabbit No. | Results of vaccination of rabbits with commercial vaccine and control in 4 dilutions, expressed as percentage of area of skin covered by confluent eruption and additional isolated lesions. The control results are placed below those for the commercial vaccine | | | | | | | | Days in cold storage before test of bulk vaccine on children |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | 1:1,000 | | 1:3,000 | | 1:10,000 | | 1:30,000 | | |
| | | | | | | | % | | % | | % | | % | | |
| M | "Auto-vacuum" freezer packed in excelsior. | 20 | 0 | 66 | 10 | 46 | 90 | | 80 | | 25 | 10 | 0 | 12 | 16 to 21 |
| | | | | | | | 95 | | 90 | | 0 | 28 | 0 | 2 | |
| N | do | 20 | 9 | 85 | 0 | 49 | 0 | 30 | 5 | 10 | 0 | 19 | 0 | 2 | |
| | | | | | | | 30 | | 80 | | 30 | | 0 | 1 | |
| | | | | | | 50 | 100 | | 100 | | 0 | 7 | 0 | 4 | |
| | | | | | | | 95 | | 95 | | 60 | | 0 | 0 | |
| O | Vacuum bottle packed in chest with can of ice. | 11 | 7 | 66 | 2 | 35 | 100 | | 100 | | 60 | | 10 | 13 | 37 to 60 |
| | | | | | | | 75 | | 75 | | 0 | 22 | 0 | 5 | |
| P | do | 11 | 37 | 85 | 13 | 57 | 100 | | 80 | | 60 | | 0 | 4 | |
| | | | | | | | 95 | | 80 | | 0 | 9 | 0 | 0 | |
| | | | | | | 58 | 80 | | 75 | | 60 | | 0 | 2 | |
| | | | | | | | 75 | | 50 | | 0 | 3 | 0 | 1 | |
| Q | Vacuum bottle, no ice | | 6 | 66 | 3 | 37 | 100 | | 98 | | 40 | 10 | 0 | 11 | 40 |
| | | | | | | | 98 | | 95 | | 40 | 13 | 0 | 6 | |
| | | | | | | 38 | 100 | | 98 | | 75 | 41 | 0 | 16 | |
| | | | | | | | 95 | | 95 | | 0 | 10 | 0 | 3 | |
| R | "Auto-vacuum" freezer packed with ice and salt, all in pail of ice. | −4 | 2 | 66 | 7 | 43 | 40 | 7 | 40 | | 5 | 7 | 0 | 1 | 33 |
| | | | | | 1:1,000 | 2 | 0 | 13 | 0 | 9 | 0 | 1 | 0 | 2 | |
| | | | | | | *44 | 100 | | 50 | | 0 | 10 | 0 | 4 | |
| | | | | | | | 50 | | 50 | | 0 | 13 | 0 | 1 | |
| S | Vacuum bottle containing ice. | | 1 | 50 | 15 | 31 | 100 | | 80 | | 60 | | 0 | 11 | 1 |
| | | | E. R. 5 | | | | 100 | | 95 | | 95 | | 50 | | E. R. 5 |
| | | | 3 | | | *32 | 0 | 29 | 0 | 15 | 0 | 1 | 0 | 0 | 30 to 36 |
| | | | | | | | 0 | 21 | 0 | 19 | 0 | 5 | 0 | 1 | |
| T | do | | 1 | 85 | 0 | 51 | 0 | 14 | 0 | 14 | 0 | 8 | 0 | 3 | |
| | | | E. R. 5 | | 1:1,000 | 1 | 0 | 15 | 30 | | 0 | 5 | 0 | 0 | |
| | | | 24 | | | 52 | 100 | | 100 | | 30 | | 0 | 3 | |
| | | | | | | | 75 | | 90 | | 0 | 8 | 0 | 3 | |
| U | do | | 1 | 85 | 11 | 55 | 25 | | 20 | | 80 | | 8 | | |
| | | | E. R. 5 | | | | 50 | | 90 | | 60 | | 30 | | |
| | | | 34 | | | 56 | 25 | | 20 | | 0 | 12 | 0 | 1 | |
| | | | | | | | 50 | | 50 | | 0 | 16 | 0 | 1 | |
| V | Vacuum bottle, no ice | | E. R. 4 | 66 | 0 | 33 | 75 | | 50 | 3 | 40 | | 0 | 5 | E. R. 4 |
| | | | 3 | | | | 50 | | 90 | | 30 | 6 | 0 | 1 | 36 |
| | | | | | | *34 | 0 | 6 | 0 | 3 | 0 | 0 | 0 | 0 | |
| | | | | | | | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | |
| W | do | | 39 | 85 | 13 | 59 | 50 | | 90 | | 50 | | 0 | 0 | |
| | | | | | | | 95 | | 90 | | 0 | 4 | 0 | 0 | |
| | | | | | | 60 | 95 | | 90 | | 30 | | 0 | 0 | |
| | | | | | | | 95 | | 95 | | 0 | 4 | 0 | 0 | |
| X | Iced container | | 30 | 66 | 10 | 47 | 100 | | 100 | | 100 | | 50 | | 59 |
| | | | | | 1:1,000 | 2 | 90 | | 90 | | 0 | 7 | 0 | 0 | |
| | | | | | | 48 | 100 | | 100 | | 85 | | 0 | 3 | |
| | | | | | | | 80 | | 50 | | 0 | 5 | 0 | 0 | |
| Y | "Auto-vacuum" freezer packed in sawdust. Freezer half full of water in which was can of vaccine. | 10 | 1 | 85 | 0 | 53 | 0 | 4 | 0 | 3 | 0 | 2 | 0 | 0 | 34 to 41 |
| | | | | | 1:1,000 | 1 | 90 | | 40 | | 0 | 12 | 0 | 3 | |
| | | | | | | 54 | 0 | 4 | 0 | 3 | 0 | 1 | 0 | 1 | |
| | | | | | | | 100 | | 75 | | 30 | | 0 | 4 | |
| Z | Vacuum bottle containing ice. | 2 | 8 | 66 | 5 | 39 | 0 | 23 | 0 | 9 | 0 | 1 | 0 | 0 | 48 |
| | | | | | | | 60 | | 50 | 6 | 0 | 12 | 0 | 1 | |
| | | | | | | *40 | 0 | 4 | 0 | 5 | 0 | 1 | 0 | 0 | |
| | | | | | | | 0 | 24 | 0 | 12 | 0 | 2 | 0 | 1 | |
| AA | do | 2 | 9 | 66 | 6 | 41 | 70 | | 65 | | 0 | 4 | 0 | 3 | 50 |
| | | | | | 1:1,000 | 1 | 95 | | 50 | | 0 | 15 | 0 | 6 | |
| | | | | | | *42 | 0 | 9 | 0 | 6? | 0 | 0 | 0 | 0 | |
| | | | | | | | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | |

[1] Untested.

NOTE.—Animals having previous contact with smallpox indicated by (*).

*smallpox vaccine received in bulk*

| Results of vaccination of children with bulk vaccine. Figures indicate number of insertions; letters indicate classes of lesions (fig. 3, lower row) | | | Days in cold storage before tubing | Days in tubes at room temperature | Days tubes in electric refrigerator before tests on children | Results of vaccination of children with tubed vaccine | | | Days tubes in electric refrigerator between tests on children and return to cold storage | Days in cold storage before shipment from Washington, D. C. to Berkeley, Calif. | Temperature, centigrade, when received | Days in commercial cold storage before tests on college entrants showing no vaccination scars | Results of tests of tubed vaccine on college entrants | | | Vaccine |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Good A B | Poor C D E | Failure F | | | | Good A B | Poor C D E | Failure F | | | | | Good A B | Poor C D E | Failure F | |
| 18 | ------ | ------ | 8 | 5 | 3 to 8 | 26 | 2 | 3 | 29 to 34 | ------ | ---- | ------ | ------ | ------ | ------ | M. |
| ------ | ------ | ------ | 8 | 5 | -------- | ---- | ------ | ------ | 37 | 57 | 20.5 | 0 | ------ | 3 | 5 | N. |
| 3 | ------ | ------ | 23 | 5 | 9 to 23 | 10 | ------ | 2 | 5 to 19 | 57 | 20.5 | 0 | 15 | 1 | 14 | O. |
| ------ | ------ | ------ | 64 | 6 | -------- | ---- | ------ | ------ | -------- | 52 | 20.5 | 0 | 30 | 9 | 45 | P. |
| 10 | ------ | 2 | 37 | 2 | 1 | 26 | ------ | ------ | 23 | 77 | 21.0 | 125 | (1) | (1) | (1) | Q. |
| 4 | ------ | ------ | 29 | 4 | 0 | 6 | 1 | 1 | 21 | 77 | 21.0 | 125 | ------ | ------ | 46 | R. |
| 5 | ------ | ------ | 1<br>E. R. 5<br>22 | 5 | 3 to 9 | 11 | ------ | ------ | 37 to 43 | ------ | ---- | ------ | ------ | ------ | ------ | S. |
| ------ | ------ | ------ | 1<br>E. R. 5<br>43 | 7 | -------- | ---- | ------ | ------ | 14 | 57 | 20.5 | 0 | ------ | ------ | 16 | T. |
| ------ | ------ | ------ | 1<br>E. R. 5<br>43 | 7 | -------- | ---- | ------ | ------ | 14 | 77 | 21.0 | 125 | ------ | ------ | 10 | U. |
| 21 | ------ | ------ | E. R. 4<br>13 | 5 | 15 | 39 | 1 | 2 | 27 | 57 | 20.5 | 0 | 13 | 3 | 14 | V. |
| ------ | ------ | ------ | 66 | 6 | -------- | ---- | ------ | ------ | -------- | 52 | 20.5 | 0 | 4 | 4 | 6 | W. |
| 3 | ------ | ------ | 53 | 5 | 1 | 6 | 2 | 1 | 21 | 77 | 21.0 | 125 | 1 | ------ | 7 | X. |
| ------ | 2 | 1 | 20 | 6 | 8 to 14 | ------ | 2 | 4 | 0 to 6 | 57 | 20.5 | 0 | 1 | 4 | 23 | Y. |
| 2 | 6 | 1 | 32 | 6 | 0 | ------ | 15 | 6 | 14 | 57 | 20.5 | 0 | ------ | 1 | 19 | Z. |
| 2 | 1 | 1 | 16 | 5 | 28 | 5 | 2 | 2 | 13 | 57 | 20.5 | 0 | 6 | 1 | 20 | AA |

# THE IMMUNOLOGICAL RELATIONSHIP OF ALASTRIM AND MILD SMALLPOX

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This paper presents the results of some experiments which tend to show the identity of an eruptive disease prevalent in tropical America with the mild smallpox of the United States and Canada. Without actual experience with the somewhat similar but variously named epidemics in different parts of the world, their specific identity or nonidentity can not be dogmatically stated. However, it would seem from the published descriptions of these variola-like eruptions that they are all of the same general character as the smallpox which is now endemic in the United States. The variola-like disease was formerly called varioloid varicella in Jamaica and Trinidad, but more recently in Jamaica and Haiti it has been called alastrim, Kafir milk pox, and amaas. The name "alastrim" was first used in Brazil, and the terms "Kafir milk pox" and "amaas" were applied to a similar eruptive fever in South Africa. Sanaga pox (in the African Kamerun) and "the Australian disease" are also to be mentioned in this connection. Cuban itch and Philippine itch have been synonymous in the United States with the mild type of smallpox which first attracted attention following the Spanish-American War.

Anderson (1867), described an epidemic under the name of varioloid varicella, in Jamaica. The eruption consisted of papules changing to semiglobular vesicles which, though confluent on the face, hands, and feet, tended to crust without going on to suppuration. Two days of fever preceded the eruption, but there was no secondary fever other than a slight intermittent elevation of temperature, thought by Anderson to be due to malaria. He states that the disease occurred in vaccinated and unvaccinated alike, but does not support this assertion by statistics. Two cases were seen in persons pitted from smallpox attacks 10 years previously. The preeruptive symptoms and the distribution of the eruption point to a diagnosis of mild smallpox rather than chicken pox.

Mild smallpox, apparently spreading from our southern negro, did not attract attention in the United States until the end of the nineteenth century. In 1896 there were only 6 deaths in an outbreak of 228 cases in four counties of Tennessee, and 38 cases with only 1

death in a Texas outbreak. In 1897 there were 2 deaths out of 200 cases reported in Alabama, and Florida reported 68 cases, 35 of which were designated as varioloid. Doubt began to be expressed as to the nature of the disease on account of the low rate of mortality. In 1898 the mild type of the disease prevailed extensively. From January 5 to March 10 there were 354 cases and 9 deaths in Birmingham, Ala., almost exclusively among the negroes. In 1899 there were 4 deaths and 323 cases in Springfield, Mo.; in 1900, 9 deaths and 801 cases in Cleveland, Ohio; in 1901, 2 deaths and 227 cases in Spokane, Wash.; in 1902, 2 deaths and 261 cases in Wayne County, Mich.

By 1900 this mild disease had attracted much attention, and many physicians refused to consider it smallpox. Happel (1900, 1901) read two papers before the American Medical Association describing the disease as it occurred among the negroes of Tennessee. He denied its identity with smallpox on the grounds of—

1. Superficial situation of the vesicles which were unicellular in form with no early umbilication.
2. Absence of secondary inflammation around the bases of the vesicles, even when confluent, and corresponding absence of secondary fever.
3. Absence of pitting; the crust fell off leaving an elevated nodule which was absorbed, leaving a residual macule.
4. Low mortality.
5. Failure of protection by vaccination.

In support of the last contention is given the history of a family of 10 persons vaccinated 9 days after exposure to the disease. In 6 persons vaccinia developed 7 days later and in 7 the smallpox eruption appeared on the 14th day after vaccination. It is obvious that in these cases vaccinia and smallpox were practically coincident in development. The ones whose vaccination failed to take—i. e., presumably immune—either escaped or had very light attacks. In the discussion which followed, Happel's stand was attacked on the grounds that a scar is not evidence of immunity, many scars being the result of infection with pathogenic organisms in a virus whose potency had passed. It was pointed out that there had been no compulsory vaccination of negroes since the days of slavery, and this would tend to build up a susceptible population group. Furthermore, absence of secondary infection was not a sufficient criterion to form the corner stone of a differential diagnosis. At the same meeting of the American Medical Association, Bracken (1901) reported that, in 662 cases of the disease, he had seen only two vaccinated successfully within the preceding six years. He saw no reason why vaccinoid, or modified vaccinia, might not follow a variola as readily as varioloid, or modified variola, might follow a vaccinia. Both depend on the degree and duration of the immunity conferred by the first infection. A

mild smallpox might not give a high degree of immunity, so a vaccinoid might easily result on subsequent vaccination of the subject. The discussion closed with the passage of a resolution declaring that the disease called pseudosmallpox in the United States was genuine smallpox and should be treated as such.

The Annual Report of the Surgeon General of the United States Public Health Service for 1911 calls attention to the two types of the disease which have coexisted in the United States in different epidemics. The virulent type has a case mortality rate (fatality) of 15 per cent or higher, while in the widely prevalent mild type, which seems to produce only occasional grave cases, the case mortality rate is usually less than 1 per cent. "Similar mild outbreaks have been reported in western Africa, in the West Indies, in certain parts of Brazil, and in Canada." All these reports of a mild disease, similar to smallpox, in dark-skinned races, except the report of Anderson (1867), were subsequent to the appearance of mild smallpox in the United States. Transitory outbreaks of unquestioned smallpox with low mortality have been known since the time of Sydenham.

A. Plehn (1902) observed the last part of an epidemic on the Sanaga River in Kamerun during 1900. The disease seemed to have affected most of the negroes but no Europeans. There was no mortality. The onset was sudden, influenza-like, with general symptoms of high fever, headache, and backache, which subsided when the eruption came out. The eruption in character of lesions and distribution resembled exactly that of smallpox, but scarring was infrequent and the dark pigmented macules remaining after the eruption disappeared within a few months. Six months after recovery 40 children of a mission school were vaccinated, and 36 gave positive reactions. Plehn does not state whether these successful vaccinations were primary vaccinias or vaccinoids in their time relations. The epidemic of Sanaga pox corresponds more closely to usual smallpox than does a peculiar, more or less afebrile, outbreak of variola which Plehn takes for the type of smallpox among West African negroes. Rudolph's (1911) attempt to differentiate alastrim from Sanaga pox is based on misquotations from Plehn.

Dickson and Lasselle (1903) reported from Trinidad an epidemic of 4,000 cases, which they called varioloid varicella. The eruption consisted of unilocular bullae with no early umbilication. These lesions dried to crusts on a firm base which contracted to form a shallow pit or a macule. Lesions in all stages were present at one time. The preeruptive symptoms and the distribution of the eruption resembled smallpox. There was no secondary fever in 95 per cent of the cases. The fatality was 0.44 per cent. Only 10 per cent of cases occurred before the age of 10. The disease was reported from Irapa, Venezuela, where it was called lechina. Vaccination of 185

persons on recovery gave 15 positive, 25 modified, and 145 negative results. Twelve cases of second attack were reported. Twenty-seven cases had been vaccinated from one to eight years previously. In the discussion which followed the paper of Dickson and Lasselle, Clarke, of Barbados, reported a simultaneous epidemic on this neighboring island. Clarke stated that there were cases of smallpox during the whole of the outbreak so typical as to leave no doubt as to the nature of the epidemic in Barbados.

De Korte (1904) described a similar disease in South Africa under the name "amaas" or "Kafir milk pox." As with "Sanaga pox," only the natives were affected, and the mortality was low. After three days of fever, with headache, backache, and coryza, the eruption appeared and the patients felt much better. There was no secondary fever, even in confluent cases, and the scars were not pitted. De Korte gives detailed case histories of 14 persons vaccinated while convalescent; all but one reacted "like persons more or less protected by previous vaccinia." De Korte thinks that the vaccinia was delayed and modified because, at the first observation, areolas were not observed, but this observation was never earlier than the seventh day. Judging from his record, most of the reactions had passed their height at the time of the first observation. It is somewhat easier to determine from his published account whether the reactions were vaccinoids or reactions of immunity than is the case with Plehn and other observers. De Korte inoculated a monkey (species not given) subcutaneously with two drops of vesicle contents. No reaction from this inoculation is recorded. Subsequently the monkey was vaccinated, with what appeared to be a vaccinoid resulting, the vesicles appearing on the fifth day, crusts on the eighth. "The result was such as one would have expected had the monkey been previously protected." De Korte concludes that amaas is smallpox modified by an undetermined factor.

In 1910 a disease called alastrim, or white pox, appeared on the Sao Francisco in the Brazilian State of Bahia and spread up this river and its tributary valleys, invading the States of Minas Geraes, Goyaz, and Sao Paulo. It was described and studied by Ribas (1910), Rudolph (1911), and de Baurepaire Aragao (1911). Ribas held that this Brazilian alastrim was comparable with South African amaas. He differentiated it from smallpox on the usual grounds of low mortality (0.5 to 2 per cent), mildness in children, lack of umbilication in the pustule, absence of secondary fever, and short duration of immunity against vaccinia. The description of Rudolph is most careful. He notes that the pustule is formed more superficially than in typical smallpox, which accounts for the absence of umbilication. The failure of secondary infection is explained by absence of streptococci in the vesicles. Rudolph saw only three cases in persons successfully

vaccinated, and these were very mild. Vaccination of convalescents gave no result in 20 persons vaccinated 1 to 6 months after recovery. Of 11 vaccinated 8 to 11 months after recovery, all failed but 3, who showed reactions similar to those of revaccination. Aragao was unable to produce eruptions by cutaneous inoculation of calves and rabbits with alastrim, subsequent vaccinations resulting positively. He considered the absence of streptococci significant in explaining the mildness of the disease. He produced vaccine pustules (time of reaction not specified) in 11 out of 19 persons vaccinated within a year after recovery. Aragao believed alastrim and chicken pox to be paravariolas.

A similar epidemic occurred in 1913 and 1914 in Australia and New Zealand. In Australia about 900 cases occurred without a death. The disease was generally considered mild smallpox. Inoculation experiments by Cleland and Ferguson (1914–15) were inconclusive. Those by Greene (1916) on calves gave equivocal results; the inoculation of monkeys produced a variola-like eruption; vaccinia protected against variola in 100 per cent of the cases, variola against vaccinia in 50 per cent of the cases, vaccinia against "Australian disease" in possibly 25 per cent of the cases, and variola against "Australian disease" in 40 per cent of the cases; no record of simultaneous controls of the reinoculations or daily observations to note possible acceleration is given.

Copeman (1920) described a small epidemic of similar character brought to England by a sailor from Mediterranean ports.

In September of 1920 the disease appeared in Haiti, presumably imported from Jamaica and was described by Melhorn (1921). The death rate at first was low, only one marasmic infant dying out of several hundred patients, but the fatality increased to a total of 9.3 per cent for the epidemic, there being reported 3,166 cases with 297 deaths from September 22, 1920, to April 21, 1921. The fever with its attendant headache and nausea lasted for three days, when the temperature fell and the rash appeared, first on the forehead, then on the face, arms, trunk, and legs, including the palms and soles in 90 per cent of the cases. The papules were hard and shotlike, with vesiculation on the fifth day and a seropurulent appearance a day or so later. There was no pitting, and the pigmented or anæmic spots which remained disappeared after four weeks.

Hastings (1921) reported an epidemic of at least 3,000 cases of mild smallpox occurring in Toronto in 1919. The first cases were notified as chicken pox; however, so many of these occurred in adults, that suspicion was aroused as to the accuracy of the diagnosis, and investigation revealed that the disease was really a mild form of smallpox. The incubation period was nearer 14 than 12 days. Only a half dozen cases showing a prodromal rash were

observed. The onset was sudden, with chill followed by fever ranging from 102° to 105°, with headache, and pain in limbs and abdomen, backache in 50 per cent of the cases, and nausea and vomiting in an equal proportion. The symptoms continued for about three days, when improvement set in, and during the subsequent 24 hours the rash appeared, first on the forehead, progressing to the lower extremities, including palms and soles. Many of the cases presented at the same time papules, vesicles, pustules, and crusts. There was no secondary fever, and the patient felt entirely well except for the local discomfort from the eruption. Umbilication was infrequent. The crusts left a red base without pitting. Eight or ten cases had a previous attack of smallpox. The mortality was very low, not a single death in the 3,000 cases being attributable directly to the smallpox.

Dr. L. M. Moody, (1921) Government bacteriologist of Jamaica, reported on the recent Jamaican outbreak of alastrim at the meeting of the Association for Medical Officers of Health of Jamaica. He estimates the number of cases at 7,000 up to April, 1921. The incubation periods were usually between 12 and 14 days. No prodromal rashes were seen. The onset was sudden. There was headache in 85 per cent of the 200 cases studied, backache in 54 per cent, pain in the limbs in 25 per cent, vomiting in 16 per cent. The eruption appeared on the third and fourth days in the customary sequence of smallpox on the various parts of the body; the constitutional symptoms abated when the rash appeared. The patient then felt perfectly well until maturation began. Secondary fever occurred in a few cases. Of 2,912 cases passing through the isolation hospital, only 13 died. The vesicles had a darkened central area, but no early umbilication before dessication. The scarring, if any took place, was superficial and was preceded by red maculo-papules after the crust had separated. Sixty convalescents were vaccinated by Moody between 2 and 12 weeks after onset. Fifteen of these gave no result. "Of the 45 who 'took' there were none who 'took' in a typical manner as compared with normal controls vaccinated with the same batches of lymph." No sign of "take" was visible before the seventh or eighth day, when papules appeared which ran a very indolent course. Inoculation of alastrim material on rabbits and calves was negative.

This review of the literature shows, in general, an agreement that this disease, by whatever name called, is no different from mild smallpox in the United States in the following particulars:

High infectivity.

Length of incubation period.

Suddenness of onset, influenza-like.

Cessation of the preeruptive symptoms when the rash appears.
Distribution of the eruption.
Infrequency among the vaccinated.

There are other particulars in which the disease has, by one author or another, been differentiated from severe smallpox:

Low fatality.
Absence of primary umbilication, and superficial location of the lesions.
Absence or infrequency of secondary fever and accompanying malaise.
Infrequency of pitted scars.
Predilection for the colored race and adults.
Lack of immunizing power against vaccinia.
Occurrence of secondary attacks.

The low fatality is coincident with relative freedom from secondary fever, often assumed to be due to infecting microorganisms which invade the deeper layers of the skin and is no more characteristic of alastrim than of the mild smallpox endemic in the United States and Canada, the fatality of which has been discussed above. The absence of secondary infection might explain the superficial character of the lesions, the absence of early umbilication, the absence or infrequence of secondary fever and accompanying malaise, and the infrequence of pitted scars.

Modern methods of refining and applying smallpox vaccine have reduced the number of pyogenic organisms, and the scars resulting from vaccination have a more superficial character than many formerly seen—that is, we have artificially developed a mild vaccinia comparable with mild smallpox.

The freeing of the virus from symbiont organisms is intentionally performed in passing vaccinia through calf and rabbit instead of continually through the human species.

With regard to the reported predilection of alastrim for children and for the colored race, an analysis of the annual rates of smallpox in 20 States shows that, in general, the low rates occur in States with efficient well-enforced vaccination laws, irrespective of the presence of a large colored element in the population. In California one-half of the cases reported by ages in 1919 occurred in children under 17 years of age, and one-quarter of the cases occurred from ages 6 to 12, inclusive. This is the direct result of the abolition, in 1911, of the requirement of vaccination as a condition of school attendance. It would appear, therefore, that the disease no longer depends on the colored race, nor on adults, for the majority of its victims, but is influenced solely by the condition of the population with respect to vaccination.

What is the explanation of the peculiar relationship between alastrim and smallpox vaccine? It has been stated that vaccinia does not protect against alastrim, yet Rudolph repeatedly saw the vaccinated persons to be the only ones spared in family groups stricken with alastrim. Of 4,226 cases of mild smallpox occurring in California in 1920, only 1 per cent had been vaccinated within the preceding five years. Of 662 cases, Bracken saw only 2 vaccinated successfully within the preceding six years. In Jamaica no cases of alastrim occurred among 500 contacts vaccinated by the medical officer of health (Moody, 1921), but cases did occur among the nonvaccinated members of families whose vaccinated members escaped the disease.

A great deal of emphasis has been placed on the fact that alastrim does not protect against vaccinia. Rudolph, however, found 28 persons vaccinated at various times during the 11 months following recovery to be immune, while 3 at the end of that time reacted with vaccinoids. The other observers state that pustules were produced, but none observed that typical primary vaccinia occurred soon after alastrim. How does this compare with the results of vaccination after typical smallpox? Bowen (1867) studied, for three years, the vaccination reactions of the personnel of the British Army. Among those showing marks of previous smallpox, perfect pustules were produced in from 32 to 45 per cent, modified pustules in from 11 to 27 per cent, and failures in from 34 to 46 per cent. Sinigar (1902) produced pustules in 40 of 45 persons showing evidence of previous smallpox. Of 29 university students giving a history of smallpox, 11 gave immune reactions, 11 vaccinoids, and 7 vaccinias when inoculated by one of the authors (J. N. F.).

The negro has been stated by Plehn (1903) to show a particularly short immunity to vaccinia and variola, but Plehn does not differentiate between vaccinia and vaccinoid. Of 23 negroes who had recovered from smallpox and showed definite scars, 17 were successfully vaccinated. Of these, 1 was vaccinated successfully within a year, 3 between 1 and 3 years, 2 between 3 and 4 years, and 2 between 4 and 5 years after the disease. Of 53 revaccinations without history of smallpox, 47 gave a positive result: the first inoculation was within 12 months in 7 cases, with 2 positive results; between 1 and 2 years in 15 cases, with all positive; between 2 and 3 years in 8 cases, 7 positive; between 3 and 4 years in 8 cases, all positive; between 4 and 5 years in 5 cases, all positive; over 5 years in 10 cases, all positive. Of 34 negro school children first vaccinated in 1893, 31 were successfully revaccinated in 1897. Of 41 first vaccinated in June, 1897, 28 were successfully revaccinated in December of the same year.

These observations show that the production of vaccine pustules, subsequent to a disease, is no argument against its variolous character.

It is entirely possible for a mild type of smallpox to produce a more transient type of immunity than a severe type would produce. It will be noted that alastrim confers complete immunity to vaccine virus for some time, followed by partial immunity. The same reasoning applies to second attacks.

## EXPERIMENTS

The following experiments[1] were undertaken to study the immunological relationship of the alastrim of the West Indies, the mild smallpox prevailing in the United States, and vaccinia.

The material was derived from two sources, through the courtesy of Prof. W. G. MacCallum, of Johns Hopkins University, and Lieut. Commander G. F. Clark of the Medical Corps of the United States Navy. In September, 1920, Professor MacCallum visited Jamaica and investigated a number of cases of alastrim. In the course of his investigation he secured pustule contents from several of the patients. Portions of the material from these patients were mixed with 0.5 per cent phenol in saline solution and sealed in small test tubes. These tubes were brought to Baltimore and placed in cold storage. On March 21, 1921, two of these tubes were secured from Professor MacCallum, brought to the Hygienic Laboratory in an iced container, and placed in storage at 5° C. On March 4, 1921, Lieut. Commander Clark, who was stationed in Haiti, mailed to the Hygienic Laboratory a sealed tube containing crusts from a patient having alastrim, together with some glass slides on which pustule contents had been dried. This material reached the laboratory on March 21, and was placed in storage at 5° C. With the material from these two sources the following experiments were performed.

### EXPERIMENT I

March 26, 1921: Two crusts from the Haitian patient were ground in a mortar with 12 drops of saline. A piece of sterile glass tubing, having an inside diameter of about 3 millimeters, was encircled by a file scratch and broken squarely off. Approximately 0.5 cubic centimeter of the suspension of crusts was drawn up into the tube and transferred to the left side of the freshly shaved backs of two *Macacus rhesus* monkeys, the tube being held perpendicularly to the tightly drawn skin and rubbed vigorously to and fro while the suspension was released a drop or so at a time. The friction was continued until a distinct reddening of the skin was observed.

The two tubes containing pustule contents from two Jamaican patients (G and H) were centrifuged, opened, and the phenolized saline pipetted off. Enough fresh sterile saline was then added to

[1] Experiments I, II, VIII, and IX have been published in Public Health Reports for June 24, 1921, vol. 36, p. 1437.

each tube to give approximately a 1 in 10 suspension of pustule contents. The right side of the back of each monkey was similarly inoculated with these suspensions, the anterior portion with suspension G and the posterior portion with suspension H.

When observed one day after inoculation, the inoculated areas were covered with serum scabs resembling a thin layer of beeswax. Accompanying the scabs were a number of reddish scratches which healed rapidly. With the exception of a slight shrinking, no change occurred in the beeswaxlike scabs until nine days after inoculation, when the following conditions were observed:

*Monkey 1.*—Right side, anterior, inoculated with Jamaican suspension G: The scab had been picked off, revealing 13 lesions, 12 grouped and one at about 3 millimeters distance. The lesion consisted of a reddish elevated base surmounted by a white circular summit having a depressed brownish center. The individual lesion measured 3 millimeters in diameter. Right side, posterior, inoculated with Jamaican suspension H: The waxy scab was still adherent. Left side, inoculated with Haitian suspension: The waxy scab was off, revealing five scattered lesions similar to those of area "G." (Plate I.)

*Monkey 2.*—Right side, anterior (Jamaican G): There was a group of seven lesions, one decidedly reddened from scratching. The scab was still adherent to area H. The inoculation of the left side with Haitian alastrim scabs had been made by means of long irregular scratches which healed promptly. The site showed a chain of eight lesions rather more elevated than above described, but with white summits and depressed brown centers. (Plate II.)

Eleven days after inoculation:

*Monkey 1.*—The lesions of the Jamaican G area were much increased in elevation and redness. The monkey had scratched off the whitish tops (vesicles) exposing craterlike depressions. Yellowish crusts were forming. The waxy scab was removed from the Jamaican H area, showing perfectly smooth skin beneath. Evidently there had been no itching, which accounts for the persistence of the scab. The Haitian area was similar in appearance to Jamaican G, but the lesions were separate.

*Monkey 2.*—The lesions on all sites resembled lesions on the corresponding sites on monkey 1.

The lesions on monkey 1 were scraped and the scrapings ground with saline. The day following the curetting, reddish scabs formed, which dropped off on the sixteenth day, leaving craterlike pits. Circular brownish crusts formed on each lesion of monkey 2 (fig. 11), which were removed on the thirteenth day, exposing craterlike depressions containing a small amount of pus.

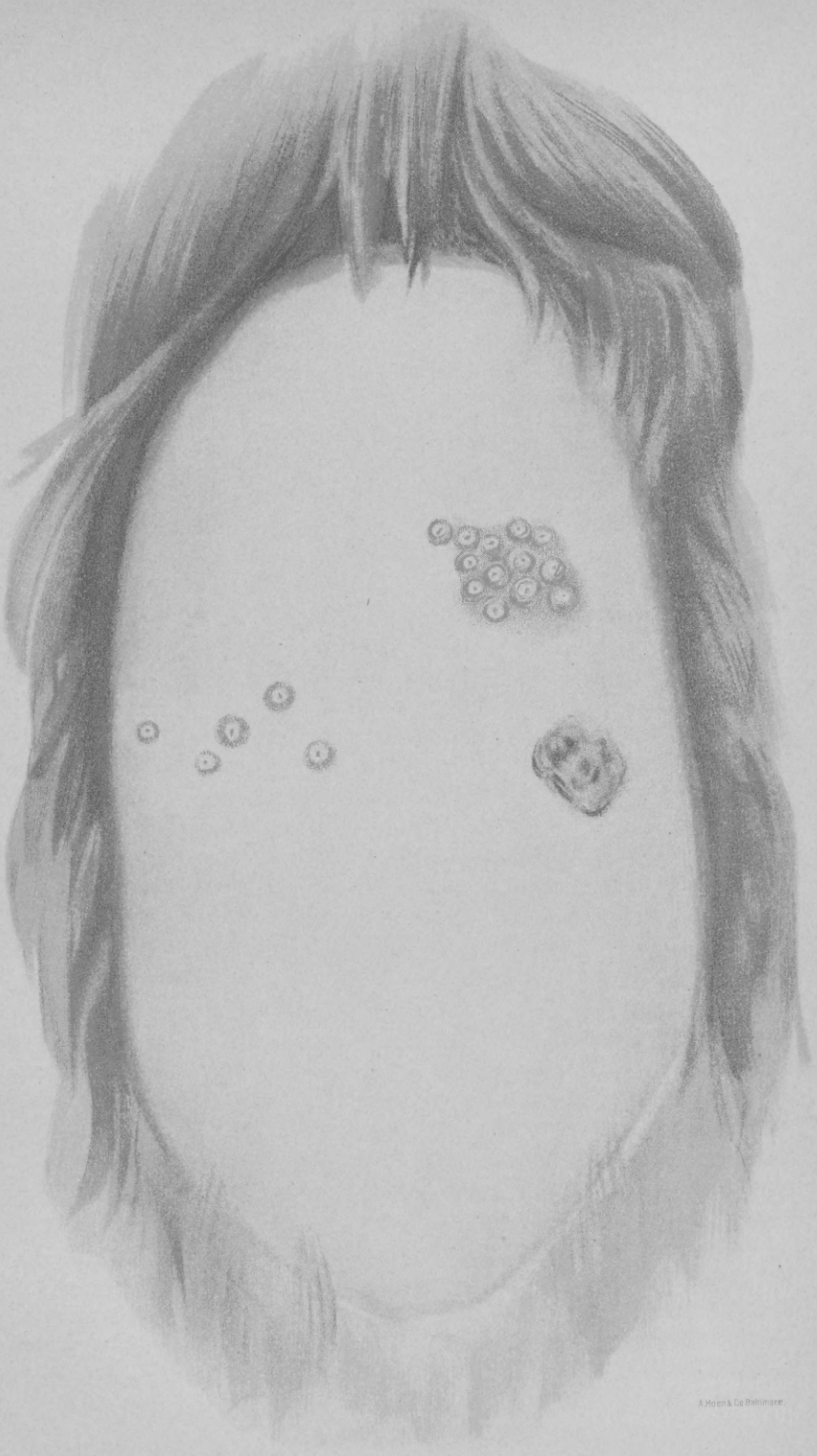

PLATE I. Monkey 1, showing alastrim lesions nine days after inoculation

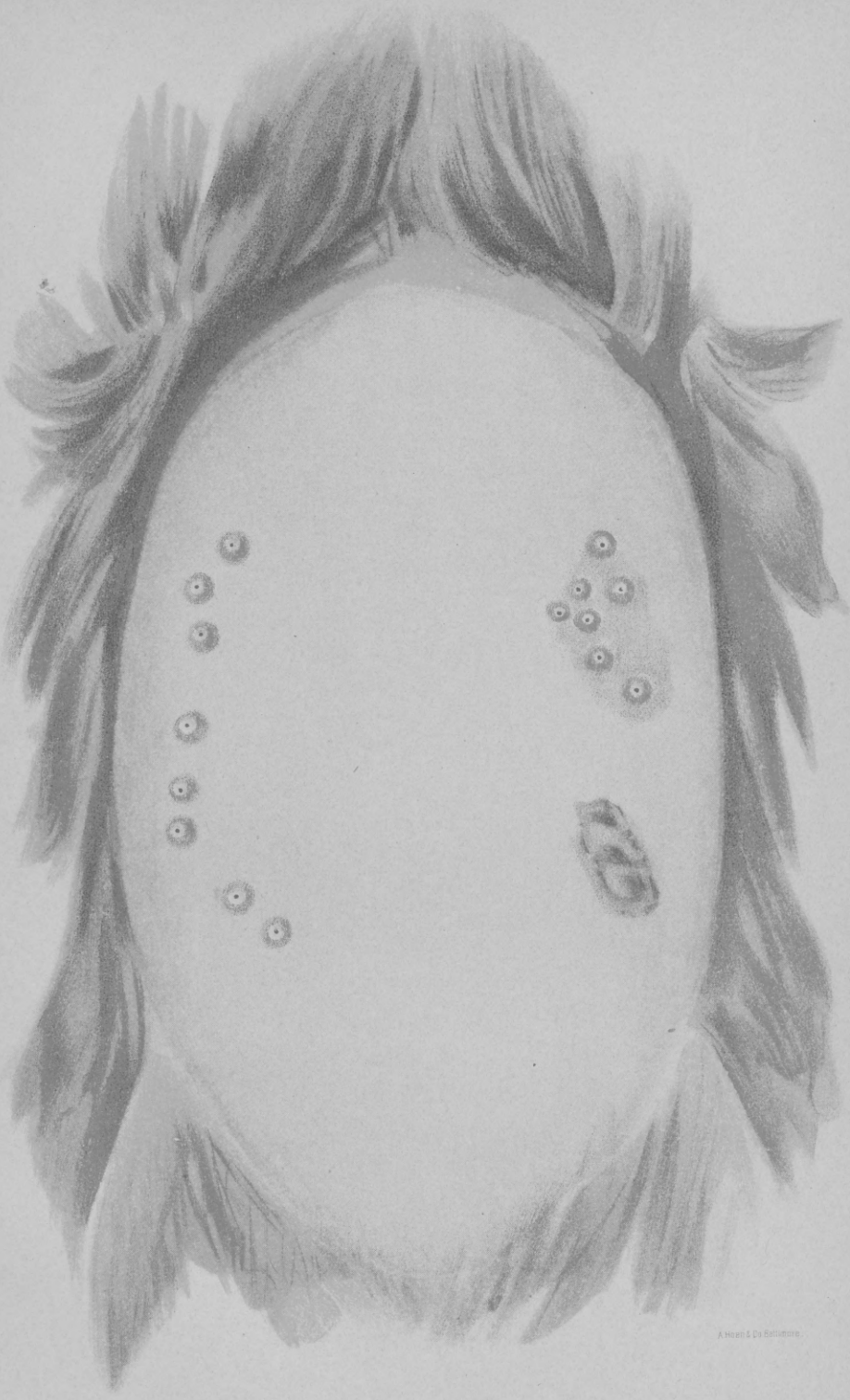

PLATE II. Monkey 2, showing alastrim lesions nine days after inoculation

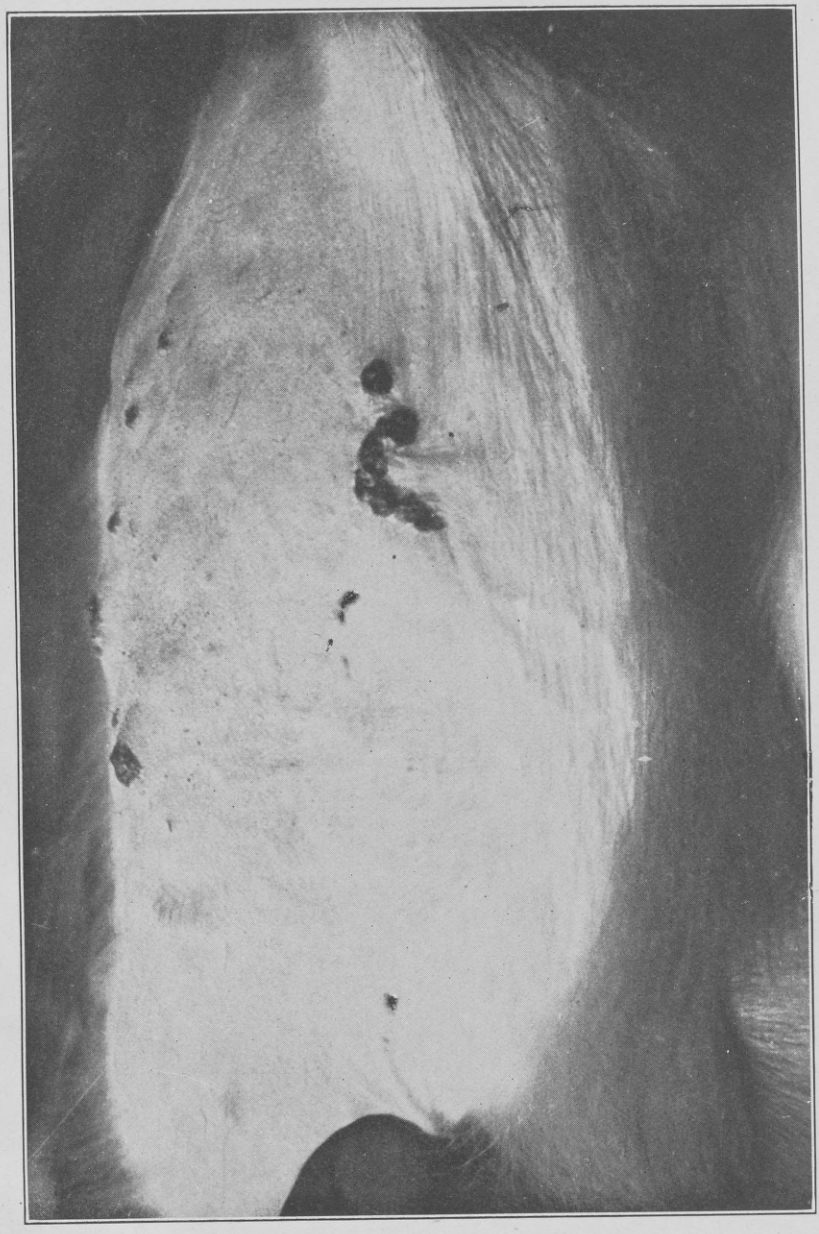

Fig. 11.—Monkey 2, showing alastrim lesions on the thirteenth day, somewhat after the height of the local eruption. The lesions on the left, shown in profile, were at the site of inoculation of crusts from the Haitian alastrim patient. The group of lesions on the right, above, resulted from inoculation with pustule contents from the Jamaican patient G. (Experiment I)

*Results.*—A vesico-papular eruption was produced by the inoculation of two monkeys with crusts from Haitian alastrim and pustule contents from Jamaican alastrim. This eruption was similar to that produced in three monkeys some months previously, inoculated by one of the authors (J. P. L.) with pustule contents from a case of smallpox occurring in the District of Columbia.

TABLE I.—*Test of immunity to vaccinia and alastrim in monkeys previously inoculated with alastrim, Experiment II*

| Monkey No. | Previous inoculations | | | | Test inoculation | |
|---|---|---|---|---|---|---|
| | Strain | Immediate source | Days prior to this test | Result | Material | Result |
| 1 | Haitian alastrim. | Human crusts | 13 | Eruption, 5 lesions covered by crust until ninth day. | Vaccine virus lot 212. | Negative, or immediate, reaction beginning first day. |
| | Jamaican alastrim G. | Pustule contents from human patient. | 13 | Eruption, 13 lesions covered by crust until ninth day. | | |
| | Jamaican alastrim H. | do | 13 | Negative. | | |
| 2 | Haitian alastrim. | Human crusts | 13 | Eruption, 8 lesions covered by crust until ninth day. | Jamaican alastrim, same pustule contents from patient G which had given positive results previously. | Negative, or early reaction first observed fifth day; disappeared seventh day. |
| | Jamaican alastrim G. | Pustule contents from human patient. | 13 | Eruption, 7 lesions covered by crust until ninth day. | | |
| | Jamaican alastrim H. | do | 13 | Negative | | |
| 4 | None | (Control) | | | Vaccine, lot 212 | Confluent vaccinia along scratches, beginning fourth day; vesicles fifth day. |

**EXPERIMENT II (Table I)**

Thirteen days after inoculation with alastrim material, the two monkeys and a normal control were inoculated as follows:

*Monkey 1.*—Inoculated with a highly potent vaccine virus in four needle scratches each about 3 centimeters in length.

*Monkey 2.*—Reinoculated with the alastrim material (Jamaican G) which had produced an eruption on both monkeys. The material was rubbed in with glass tubes as described in the first experiment.

*Monkey 4.*—A normal animal was inoculated in seven needle scratches with the potent vaccine virus used on monkey 1.

One day after inoculation:

*Monkey 1.*—The vaccination scratches were slightly elevated, but there was no redness.

*Monkey 2.*—A waxy scab had formed at the inoculation site.

*Monkey 4.*—The vaccination scratches had almost healed, there being no elevation or redness.

Three days after inoculation:

*Monkey 1.*—The scratches had healed, there being no elevation or redness.

*Monkey 2.*—No change.

*Monkey 4.*—No change.

Four days after inoculation.

*Monkey 1.*—Slight papule at end of one scratch.

*Monkey 2.*—No change.

*Monkey 4.*—The vaccination scratches showed distinct papules measuring 4 millimeters across the line of scratch, with areolæ of 5 millimeters.

Five days after inoculation:

*Monkey 1.*—The scratches were no longer palpable.

*Monkey 2.*—Part of the waxy scab was removed, exposing a skin area slightly roughened, but with no signs of inflammation.

*Monkey 4.*—Vesicles were beginning to form on the summits of the papules.

Six days after inoculation:

*Monkey 1.*—No change.

*Monkey 2.*—All the waxy scab had come off, exposing two red spots 2 millimeters in diameter, the rest of the area being smooth.

*Monkey 4.*—All seven scratches showed papules composed of overlapping circles with vesicles beginning at the summits.

Eight days after inoculation:

*Monkey 1.*—No change.

*Monkey 2.*—Small red spots fading.

*Monkey 4.*—There was definite vesicle formation over the entire papular area.

Ten days after inoculation:

*Monkey 1.*—No change. (Fig. 12.)

*Monkey 2.*—There was a slight roughness of the skin as if papules had formed and disappeared. (Fig. 13.)

*Monkey 4.*—Brownish scabs were beginning to form in the center of the vesicles. (Fig. 14.)

*Results.*—A monkey successfully inoculated with Haitian and Jamaican alastrim was refractory to a strain of vaccine virus which gave a typical vaccinia in a normal monkey. A monkey successfully inoculated with Haitian and Jamaican alastrim was refractory to the same Jamaican alastrim.

On April 28, 1921, smallpox crusts were secured from a patient (M) in the isolation hospital of the District of Columbia, and on May 20 crusts were secured from a second patient (N) in the same institution. The crusts were placed in sterile glass-stoppered bottles at the time of collection and subsequently were placed in cold storage. On May 23, 1921, Prof. W. G. MacCallum, of Johns Hopkins Medical School,

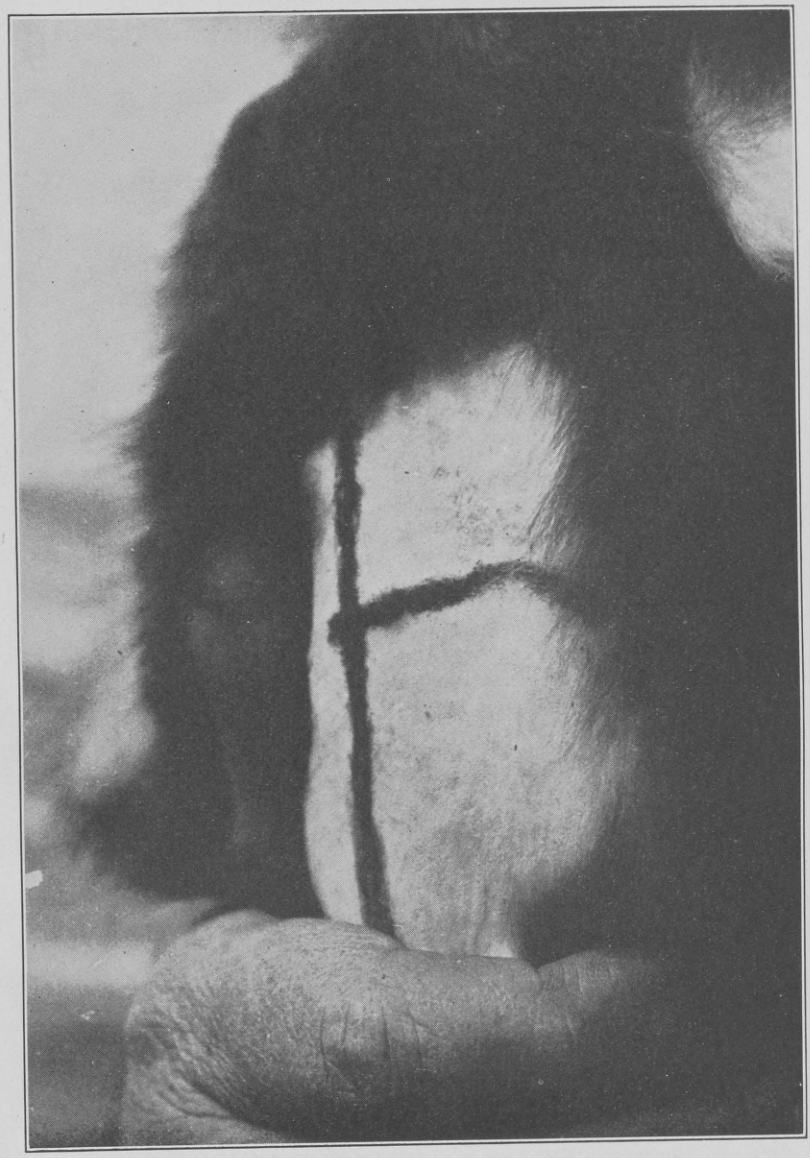

Fig. 12.—Monkey 1, showing immunity to vaccine virus (vaccinia) resulting from inoculation with the alastrim. This monkey had been inoculated 23 days previously with Haitian alastrim on the left, and with Jamaican alastrim on the right above, with successful results similar to those obtained in the case of Monkey 2 (Fig. 11). Ten days previous to this photograph, potent vaccine virus was inoculated on the area below on the right; no lesions resulted other than those of trauma or a very much accelerated reaction. The normal monkey vaccinated at the same time, as a control, had a typical vaccinia (Fig. 14). (Experiment II)

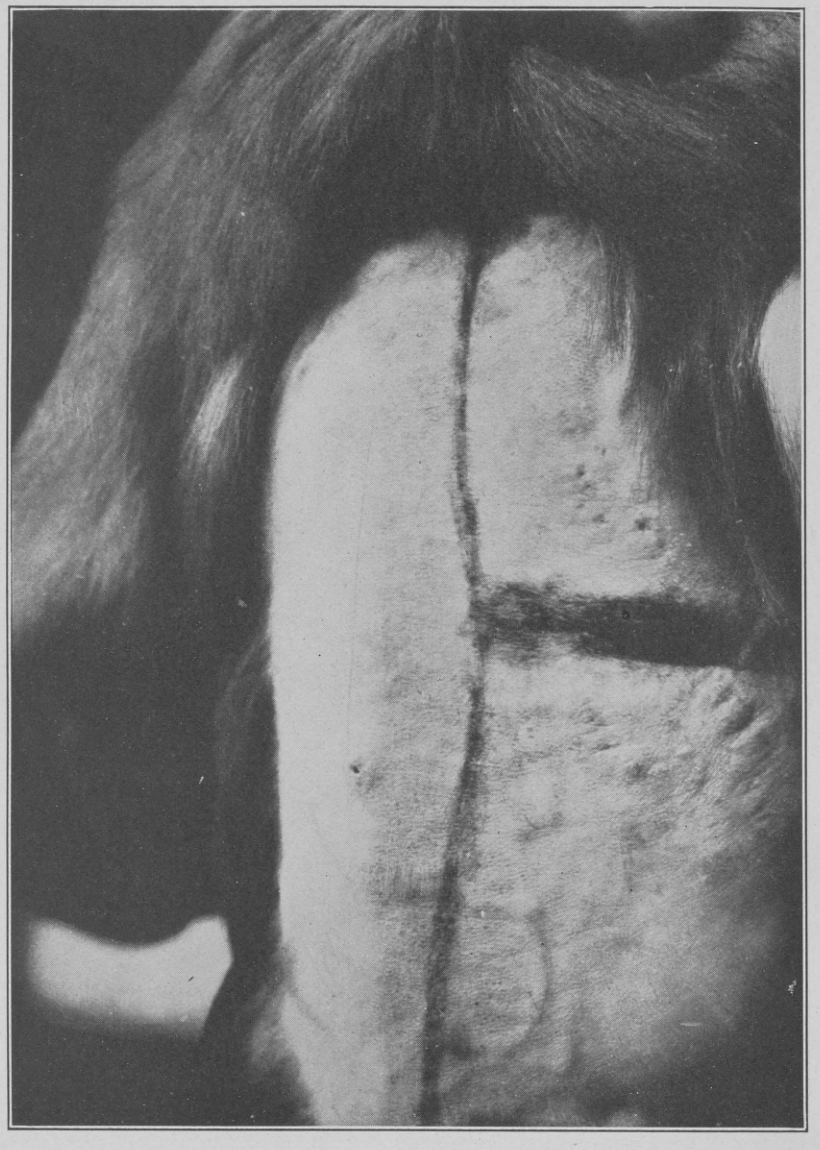

Fig. 13.—Monkey 2, showing failure to produce characteristic primary lesions when reinoculated with alastrim material. The areas on the left, and above on the right, show pits from successful alastrim inoculations 23 days previously (see Fig. 11). Below on the right, alastrim material from Jamaican patient G was reinoculated 10 days previously with no visible results other than trauma or a much accelerated reaction. (Experiment II)

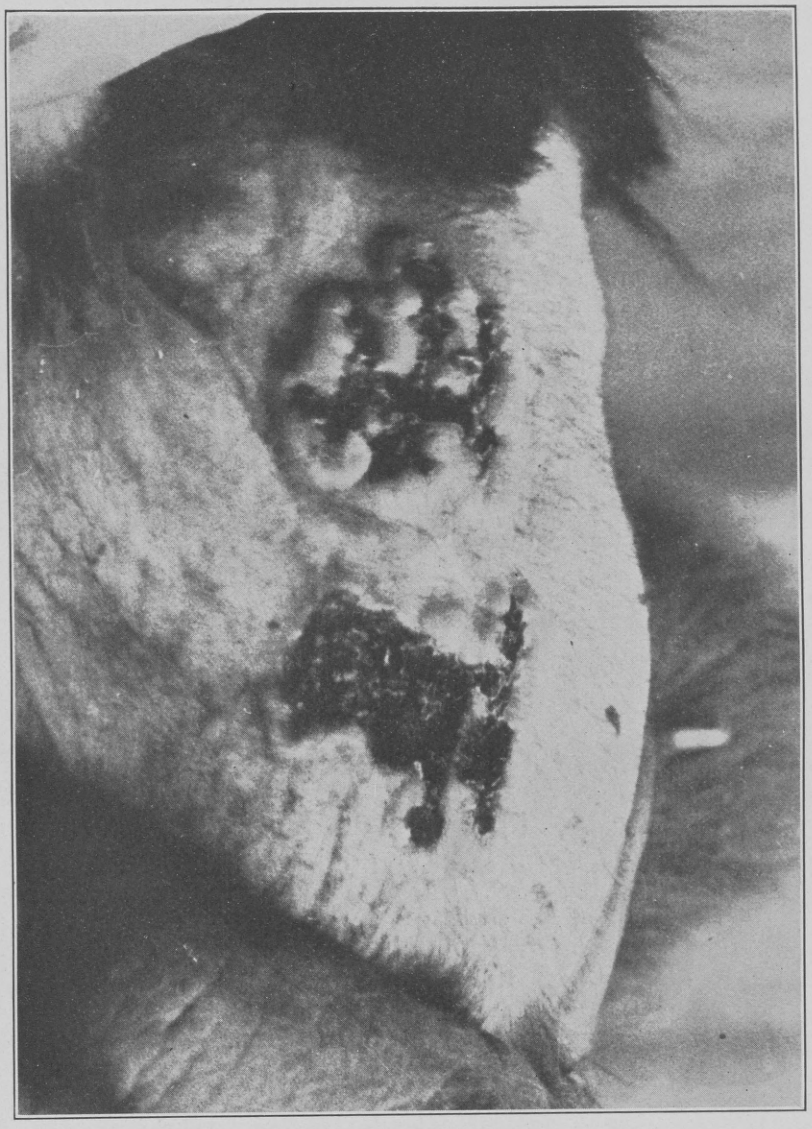

Fig. 14.—Monkey 4, showing typical vaccinia on the tenth day. This monkey, serving as a control for Monkey 1 (Fig. 12), was inoculated at the same time and in the same way. (Experiment II)

supplied sealed tubes of Jamaican alastrim pustule contents collected in September, 1920, from patients L, F, and D, mixed with phenolized saline. He also advised that the pustule contents in the five tubes furnished to date had been collected as follows: G and H on the eighth day of the eruption, L on the sixth day, F on the fifth day, and D on the seventh day.

EXPERIMENT III

May 27, 1921: The backs of four *Macacus cynomolgus* monkeys were shaved and inoculated with the following materials:

SPM—emulsion of crusts from smallpox patient M.

SPN—emulsion of crusts from smallpox patient N.

H(M2)—Haitian alastrim crusts collected from monkey 2. (Experiment I.)

JG(M2)—Jamaican G alastrim crusts collected from monkey 2. (Experiment I.)

JG—pustule contents from Jamaican patient G.

JL—pustule contents from Jamaican patient L.

JF—pustule contents from Jamaican patient F.

Tubes containing pustule contents from patients L and F were opened at the time of inoculation. The tube from patient G had been opened at the time of Experiment I. When the tubes were opened, the phenolized saline was pipetted off and sterile saline was added, about three times as much saline as sediment being used. The crusts were ground to powder and saline added to make a thick emulsion. The inoculations were made by friction with blunt pipettes cut from glass tubing, as in Experiment I.

*Monkey 5.*—Right side of back was inoculated with SPM, left side with SPN.

*Monkey 6.*—Inoculated in the same manner as monkey 5.

*Monkey 7.*—Right side of back was inoculated with H(M2), left side with JG(M2).

*Monkey 8.*—Right side of back, anterior region, was inoculated with H(M2), central region with JG, posterior region with JF. Left side of back, anterior region, was inoculated with JG(M2), central region with JL; posterior region not inoculated.

Twenty-four hours after inoculation:

*Monkey 5.*—Area inoculated with SPM has thin yellow crust, dark brown at the bottom, measuring 30 by 40 millimeters. Area inoculated with SPN has a similar crust 15 by 40 millimeters.

*Monkey 6.*—Area inoculated with SPM has thin yellow crust measuring 30 by 30 millimeters. Area inoculated with SPN has a serum crust 10 by 10 millimeters.

*Monkey 7.*—Area inoculated with H(M2) has a thin crust measuring 10 by 30 millimeters, with adjacent scratches. Area inoculated with JG(M2) is reddish and denuded of epithelium.

*Monkey 8.*—Area inoculated with H(M2) shows a thin serum crust measuring 30 by 40 millimeters. Area inoculated with JG(M2) shows a thin serum crust measuring 40 by 40 millimeters with a dozen red scratches. Area inoculated with JG has the same appearance but fewer scratches. Area inoculated with JL is reddened and partly covered with serum crust. Area inoculated with JF has serum crust measuring 15 by 30 millimeters.

Forty-eight hours after inoculation:

*Monkey 5.*—Crusts slightly thicker.

*Monkey 6.*—SPM: Area has somewhat browner crust. SPN: Area covered with thin brownish crust; the marginal skin is slightly red.

*Monkey 7.*—H(M2): Area has a thin, brownish crust. JG(M2): Area has red crusts.

*Monkey 8.*—All crusts generally cleaner.

Three days after inoculation:

*Monkey 5.*—No change.

*Monkey 6.*—Redness has disappeared, leaving only brown crusts.

*Monkey 7.*—H(M2): Shows no change. JG(M2): Area shows red crusts slightly raised and contracted.

*Monkey 8.*—Scratches healing.

Four days after inoculation:

*Monkey 5.*—No change.

*Monkey 6.*—No change.

*Monkey 7.*—No change.

*Monkey 8.*—No change.

Five days after inoculation:

*Monkey 5.*—SPM: Shows thin, waxy crust with dark red band at bottom. SPN: Shows no change.

*Monkey 6.*—SPM: Area shows a waxy crust which seems to be roughened or more elevated. SPN: Shows a contracting crust.

*Monkey 7.*—H(M2): The scratches are healed; the crust is contracting. JG(M2): Shows contraction of red crusts.

*Monkey 8.*—All waxy scabs contracting on areas H(M2), JG, and JF. Scratches healing, crusts redder on JG(M2) and JL.

Six days after inoculation:

*Monkey 5.*—No change except crusts slightly thicker.

*Monkey 6.*—SPM: The crust is off; no lesion beneath. SPN: The crust is loosening.

*Monkey 7.*—H(M2): Crust thicker than four days ago. JG(M2): Crusts same as H(M2).

*Monkey 8.*—Crusts generally thicker; on the area JG(M2) and JL they are redder; apparently have been scratched and have been partially removed.

Seven days after inoculation:

*Monkey 5.*—SPM: Crust removed. SPN: Crust removed, revealing pinkish area with no apparent papulation.

*Monkey 6.*—SPM: No change. SPN: The crust is off and there is slight pinkish papulation at lateral edge of site.

*Monkey 7.*—Thick crusts; no evidence of eruption.

*Monkey 8.*—JF: Part of crust removed; two bleeding points beneath, very slight papulation anteriorly, some pinkness here and where crust was removed. JG: Thick crust. H(M2): Thick crust. JL: Pink papular eruption over that part of surface not covered by crusts. JG(M2): Thick crust.

Eight days after inoculation:

*Monkey 5.*—SPM: No change. SPN: Two papules on left anterior portion.

*Monkey 6.*—SPM: No change. SPN: Slightly reddish papules.

*Monkey 7.*—Apparently no change.

*Monkey 8.*—Papulation on JL and JF. Crusts on other areas.

Ten days after inoculation:

*Monkey 5.*—SPM: One or two slightly reddened spots. SPN: Large brown scab formed on papules.

*Monkey 6.*—SPM: In direct sunlight three small pinkish elevations appear. SPN: Papules slightly more elevated, crusts beginning.

*Monkey 7.*—Scab edges loose as if they had been scratched. Some pinkness under JG(M2).

*Monkey 8.*—Crusts appearing on JL and JF.

Eleven days after inoculation:

*Monkey 5.*—SPM: One spot has become a red papule; the other shows a flattened crust. SPN: There is a group of three crusts, one circular in form.

*Monkey 6.*—SPM: Scabs forming on papules. SPN: Several tiny brownish crusts and a pit where crust has been removed.

*Monkey 7.*—H(M2): Scab removed, revealing smooth skin. JG(M2): Scab removed exposing papule, red, slightly umbilicated, smooth, drying, but with no thick crust.

*Monkey 8.*—JF: In an area of about 15 by 30 millimeters, papules are thickly set. These are crusting with a very rough, greenish brown appearance, not waxy like the serum crust following inoculation. JG: The scab is off; the skin is smooth beneath. H(M2): The scab is off, the skin smooth. JG(M2): Two crusting papules 6 by 4 millimeters and 6 by 3 millimeters in size. JL: Area of 40 by 40 millimeters in size, resembles JF in all respects. If crusts are removed from JL or JF, bleeding or purulent points are revealed.

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Twelve days after inoculation:

June 8, 1921: All lesions practically as on preceding day. Monkey 8 photographed. (Fig. 15.) Crusts removed from all lesions, exposing in each instance craterlike depressions. All crusts from same source combined, ground, and mixed with saline.

*Results.*—Smallpox crusts (patient M) inoculated 29 days after collection on two monkeys (monkeys 5 and 6) produced two crusted papules on one monkey and three on the other. Smallpox crusts (patient N) inoculated on two monkeys (monkeys 5 and 6) seven days after collection produced three crusted papules on one monkey and about five similar lesions on the other. Crusts from an eruption produced on a monkey (monkey 2) by crusts used in Experiment I from a patient with Haitian alastrim produced no eruption on two monkeys (monkeys 7 and 8) inoculated 49 days after collection. Pustule contents used in Experiment I from a Jamaican alastrim patient (G) produced no eruption on a monkey (monkey 8). The tube containing this material had been open for two months, though kept in the ice box. Crusts from an eruption which was produced on a monkey (monkey 2) by pustule contents from this same patient (G), when inoculated on two monkeys (monkeys 7 and 8), 49 days after collection, produced one papule on one monkey and two on the other. Pustule contents from two patients (patients F and L) with Jamaican alastrim, which had been sealed in tubes with phenolized saline from September, 1920, to May 27, 1921, gave confluent crusted pustules on a monkey (monkey 8).

#### EXPERIMENT IV

June 8, 1921: The back of a *Macacus cynomolgus* monkey was shaved and scarified with a Japanese scarifier. On the right side, two cross scarifications were made; on the left side, two linear scarifications. The saline suspension of crusts collected at the end of Experiment III were inoculated as follows: Right side, anterior area, SPM(M5–6)—crusts from monkeys 5 and 6, produced by inoculation with crusts from smallpox patients M and N. Right side, posterior area, JF(M8)—alastrim crusts from eruption on monkey 8, produced by inoculation with pustule contents from Jamaican patient F. Left side, anterior area, JG(M7–8)—alastrim crusts from the eruptions on monkeys 7 and 8, produced by inoculation with crusts from an eruption on monkey 2, produced by inoculation with pustule contents from Jamaican patient G. Left side, posterior, JL(M8)—alastrim crusts from an eruption on monkey 8, produced by inoculation with pustule contents from patient L.

Twenty-four hours after inoculation:

*Monkey 9.*—SPM–N(M5–6): Not deeply scarified; shows very few scratches or discolorations. JF(M8): Thin yellowish crust through which pattern of red scratches appears. JG(M2 to M7–8):

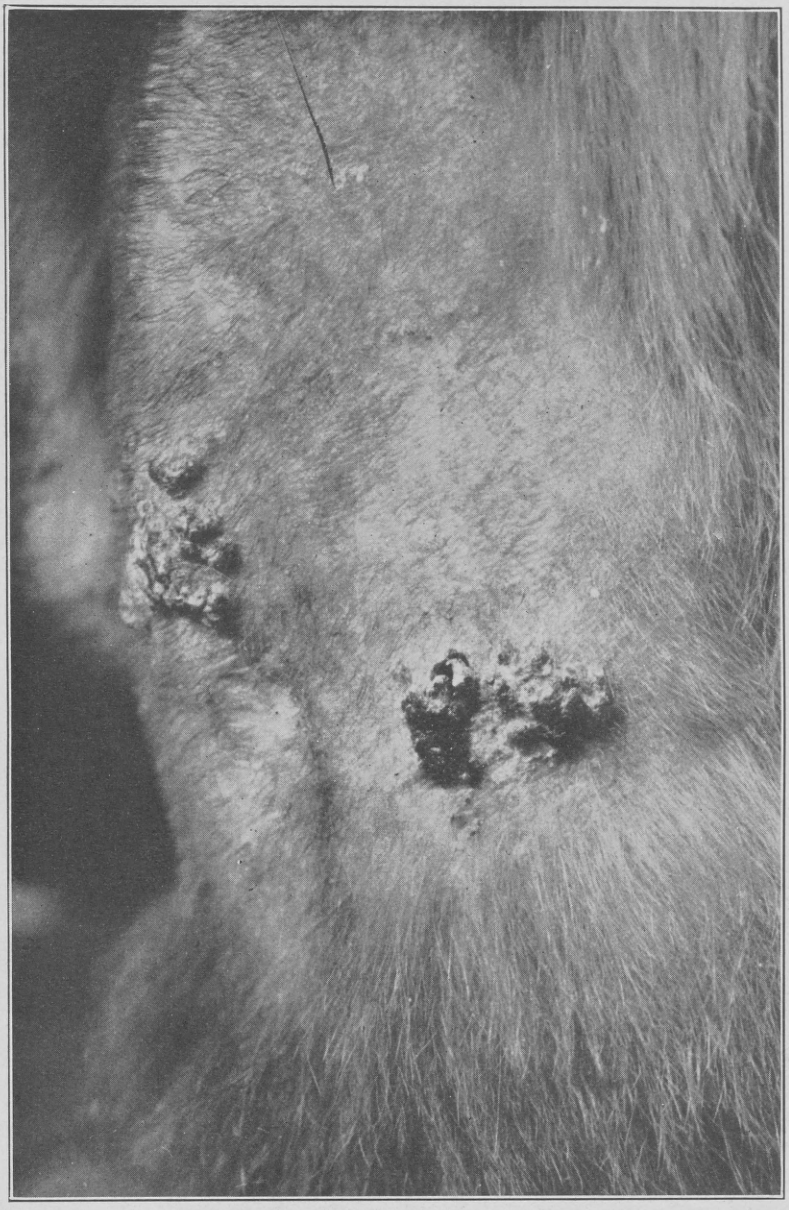

Fig. 15.—Monkey 8, showing lesions on the twelfth day after inoculation with alastrim material. On the left are the lesions resulting from the inoculation of material from Jamaican patient L, and on the right are similar lesions resulting from the inoculation with material from Jamaican patient F. (Experiment III)

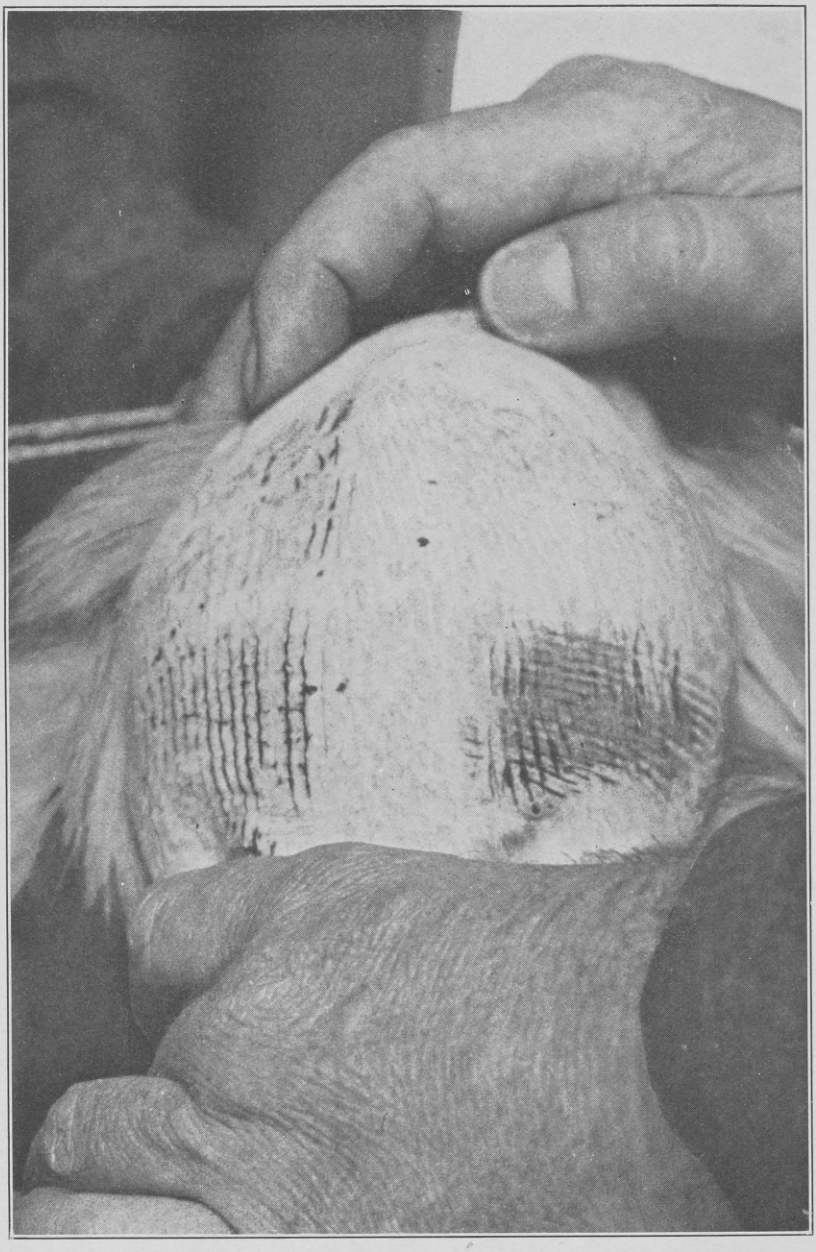

Fig. 16.—Monkey 9, five days after inoculation, showing earliest evidence of activity. Papules are forming along and outside the scratches. This monkey was inoculated on the upper right side of the back with smallpox material collected from Monkeys 5 and 6 of Experiment III. On the upper left back, this monkey was inoculated at the same time with alastrim material consisting of crusts from the areas on Monkeys 7 and 8, which had been inoculated with alastrim material from Monkey 2 (Jamaican G). Human alastrim material from Jamaican patients L and F was inoculated on the lower left and right sides respectively. Compare with Figs. 17, 18, 19, 20, and 21 for further progress of these lesions. (Experiment IV)

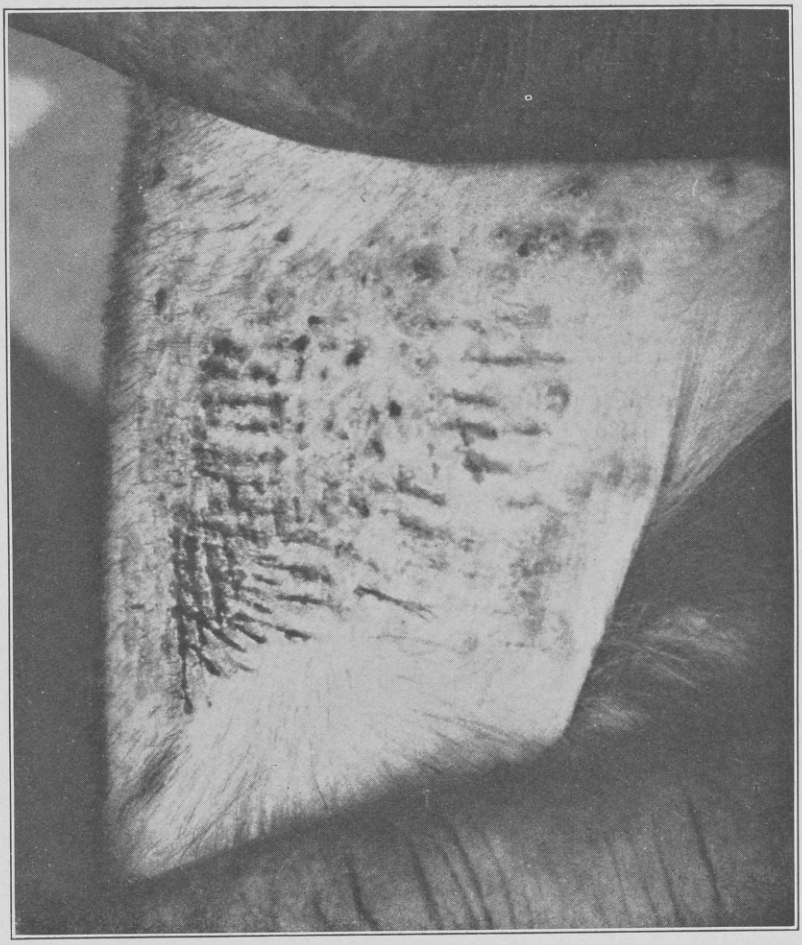

Fig. 17.—Monkey 9, seven days after inoculation, showing beginning vesiculation and umbilication in inoculated alastrim. The area shown is that inoculated with the pustule contents of Jamaican patient F. Compare with Figs. 16, 18, 19, and 21. (Experiment IV)

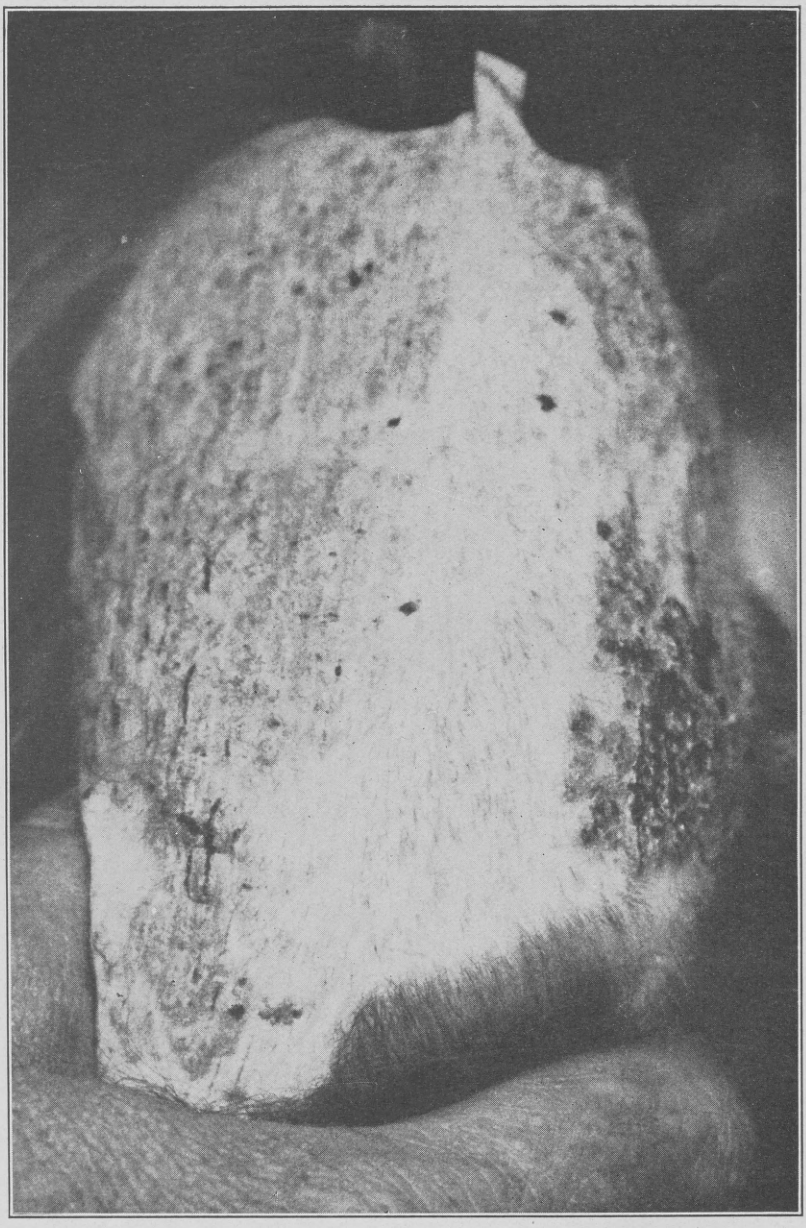

Fig. 18.—Monkey 9, eight days after inoculation. The umbilication on the lower left back inoculated with material from Jamaican patient L is clearly shown. Compare with Figs. 16, 17, 20, and 21. (Experiment IV)

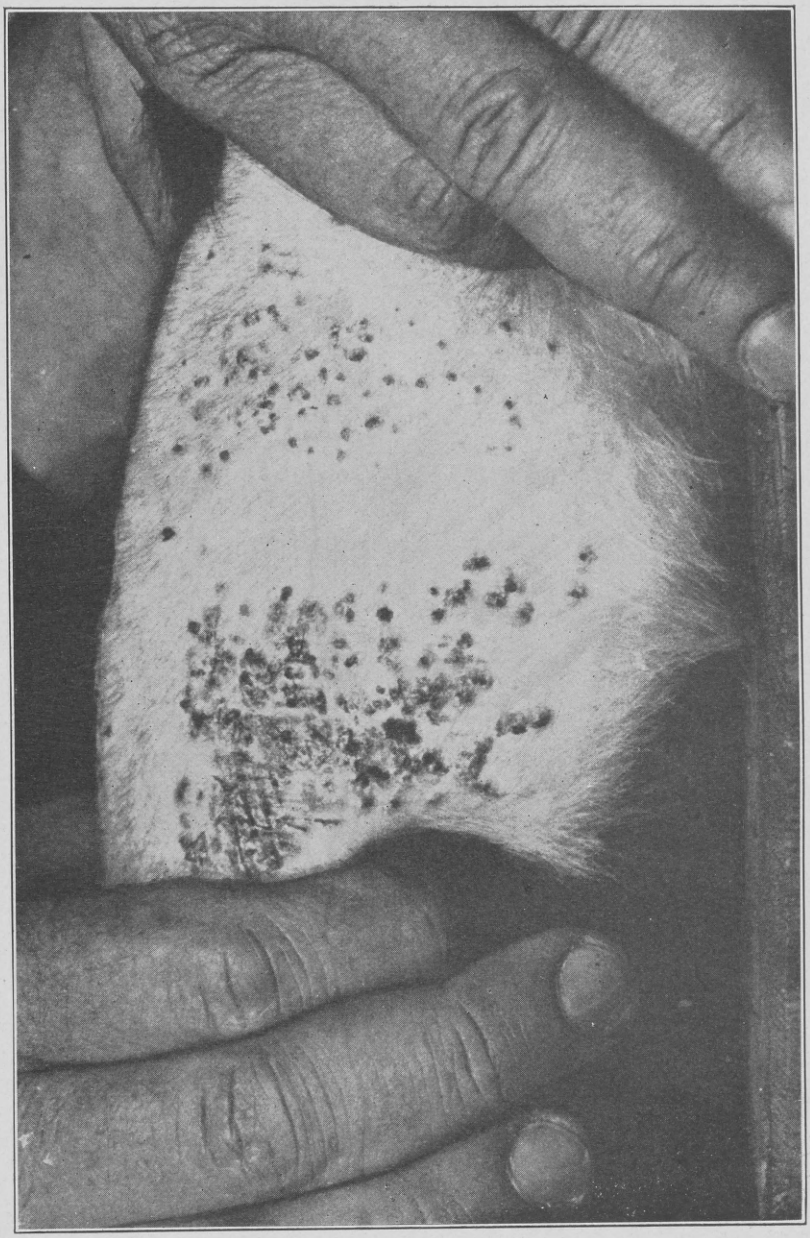

Fig. 19.—Monkey 9, nine days after inoculation. This shows the right side, which has been inoculated above with smallpox material, after one monkey passage, and below with alastrim material, also after one monkey passage. Compare with Figs. 16, 17, 18, and 21. (Experiment IV)

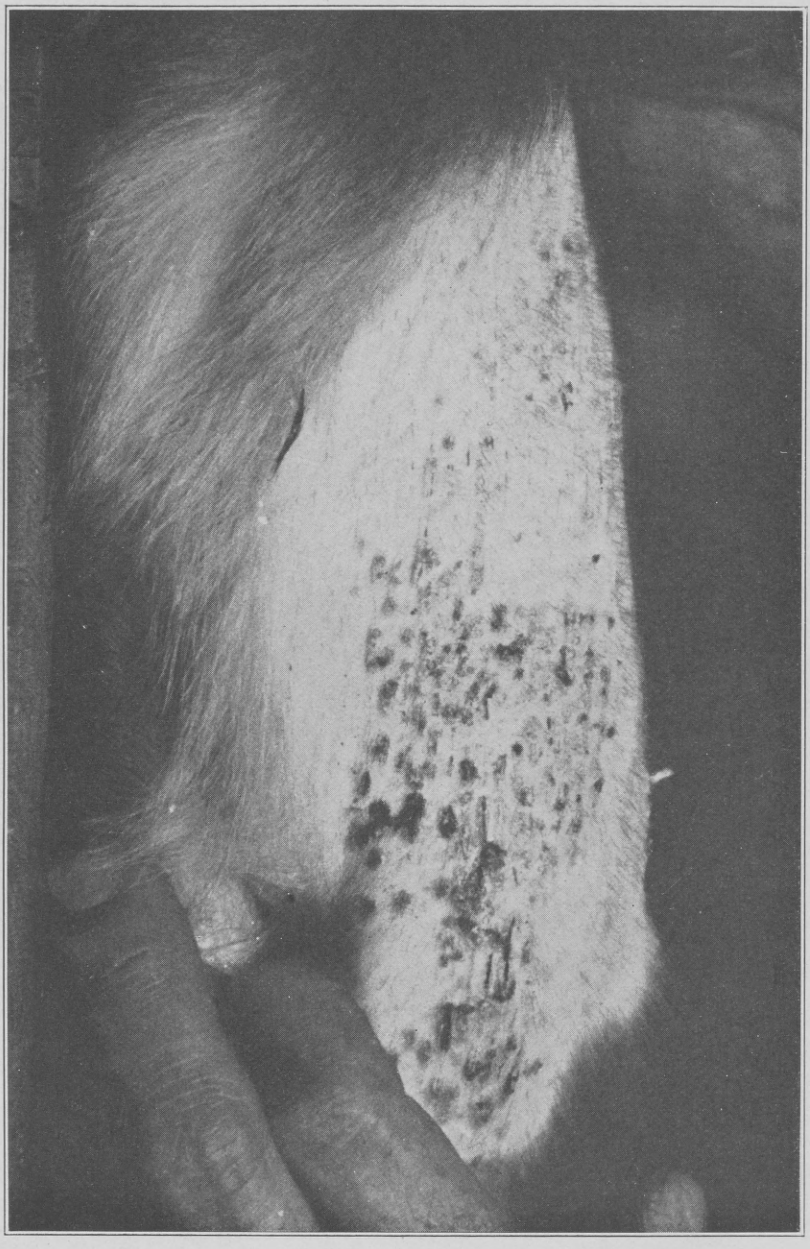

Fig. 20.—Monkey 9, nine days after inoculation. The photograph shows the left side, the upper area having been inoculated with material derived originally from Jamaican alastrim patient G and passed through two monkeys successively. The lower area was inoculated with Jamaican L strain, after one monkey passage. Compare with Figs. 16, 18, and 21. (Experiment IV)

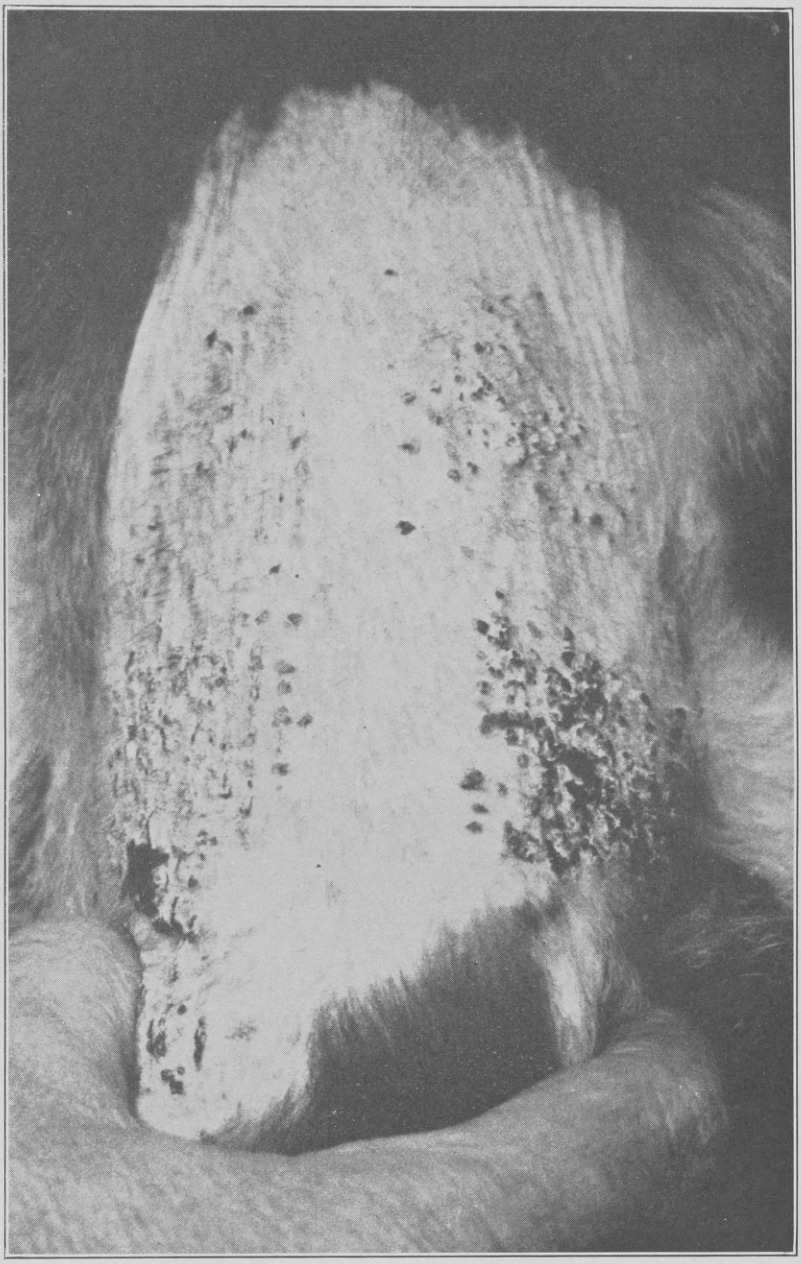

Fig. 21.—Monkey 9, ten days after inoculation. All four areas are shown as in Fig. 16. The third monkey passage of a Jamaican alastrim strain (G), and a West Virginia smallpox strain, second monkey passage above. Below are the second monkey passages of two other Jamaican alastrim strains, L and F. Compare with Figs. 16, 17, 18, 19, and 20. (Experiment IV)

Linear scratches, but no crusting nor discoloration. JL(M8): Linear scratches now browning.

Forty-eight hours after inoculation:

*Monkey 9.*—No change, except that crust on JF(M8) looks thinner and redder. All scratches are healing, having turned a darker red.

Three days after inoculation:

*Monkey 9.*—All areas yellowish brown like JF(M8).

Five days after inoculation:

*Monkey 9.*—SPM–N(M5–6): Toward median line, fairly thickly set, discrete eruption, faintly pinkish papules against blue background of skin, 1.5 to 2 millimeters in diameter. JF(M8): Area covered by very thin brownish crust, at edges of which, in healed scratches, an eruption similar to the above appears, except that lesions are more thickly set and measure 2 to 3 millimeters in diameter. JG(M2 to M7–8): No eruption. JL(M8): Papular eruption, lesions 1.5 to 2 millimeters in diameter. Monkey photographed. (Fig. 16.)

Six days after inoculation:

*Monkey 9.*—SPM–N(M5–6): Papules larger and pinker, a few show yellowish centers. JF(M8) and JL(M8): Papules larger and pinker. JG(M2 to M7–8): A few small papules appearing especially at margins of area.

Seven days after inoculation:

*Monkey 9.*—SPM–N(M5–6): More lesions have yellowish centers. JF(M8): Papules are larger, show beginning umbilication with small central brownish dot. (Fig. 17.) JG(M2 to M7–8): Papules in upper right corner of area pinker and larger. JL(M8): Not much change.

Eight days after inoculation:

*Monkey 9.*—Vesiculation and increased areola formation on all areas Eruption most discrete on JG(M2toM7–8). The tops have been removed from several of the lesions on JF(M8) showing craterlike, blood-filled spots about 1 millimeter in diameter. Monkey photographed. (Fig. 18.)

Nine days after inoculation:

*Monkey 9.*—Yellow crusts on all lesions; abrasions healing on JF(M8) (fig. 19); scratch marks, with bleeding points, on JL(M8) (fig. 20).

Ten days after inoculation:

*Monkey 9.*—Eruption on areas as follows: SPM–N(M5–6): 60 discrete lesions. JF(M8); Eruption 60 per cent confluent on scarified area. JG(M2toM7–8): 19 discrete lesions. JL(M8): Eruption 70 per cent confluent along linear scratches. Monkey photographed. (Fig. 21.) Lesions curetted, revealing pits. Collections ground separately and mixed with saline.

*Results.*—Crusts collected from approximately 13 crusted papules which were produced on two monkeys (monkeys 5 and 6) by inoculation with smallpox crusts from two patients (N and M), produced

60 vesicles when immediately inoculated on a monkey (monkey 9). Crusts collected from 3 crusted papules which were produced on two monkeys (monkeys 7 and 8) by crusts from a monkey (monkey 2) inoculated with alastrim pustule contents from patient G, produced 19 vesicles when immediately inoculated on a monkey (monkey 9). Crusts collected from confluent eruptions which were produced on a monkey (monkey 8) by alastrim pustule contents from patients F and L gave eruptions 60 and 70 per cent confluent, respectively, when inoculated immediately on a monkey (monkey 9).

TABLE II.—*Test of immunity to vaccinia in monkeys previously inoculated with alastrim or smallpox, Experiment V*

| Monkey No. | Previous inoculations | | | | Test inoculation | |
|---|---|---|---|---|---|---|
| | Strain | Immediate source | Days prior to this test | Result | Material | Result |
| 1 | Haitian alastrim | Human crusts | 79 | Eruption, 5 lesions, covered by crust until ninth day | Vaccine virus, lot 587 | Vaccinoid, beginning second day, vesicles third day. |
| | Jamaican alastrim G | Pustule contents from human patient | 79 | Eruption, 13 lesions covered by crust until ninth day | | |
| | Jamaican alastrim H | do | 79 | Negative | | |
| | Vaccine virus | Lot 212 | 66 | do | | |
| 6 | Smallpox N | Human crusts | 17 | Eruption, 8 lesions, beginning seventh day | do | Vaccinoid, beginning second day, vesicopustules, third day. |
| | Smallpox M | do | 17 | Eruption, 3 lesions, beginning tenth day | | |
| 8 | Jamaican alastrim G | Crusts from monkey 2 | 17 | Eruption, 2 lesions, covered by crust until eleventh day | do | Negative, or immediate reaction, beginning first day. |
| | Jamaican alastrim L | Pustule contents from human patient | 17 | Eruption, confluent, beginning seventh day | | |
| | Haitian alastrim | Crusts from monkey 2 | 17 | Negative | | |
| | Jamaican alastrim G | Pustule contents from human patient | 17 | do | | |
| | Jamaican alastrim F | do | 17 | Eruption, confluent, beginning seventh day | | |
| 10 | None | (Control) | | | do | Confluent vaccinia along scratches, beginning third day, pustules fifth day. |

**EXPERIMENT V (Table II)**

June 13, 1921: One *Macacus rhesus* monkey and three *Macacus cynomolgus* monkeys were inoculated with a potent smallpox vaccine in three needle scratches about 20 millimeters apart and 50 millimeters in length.

*Monkey 1.*—Had been successfully inoculated 79 days previously with Jamaican alastrim (G) and Haitian alastrim. (Experiment I.) Unsuccessfully inoculated with potent smallpox vaccine 13 days later. (Experiment II.)

*Monkey 6.*—Eight lesions had been produced by inoculation with smallpox crusts 17 days previously. (Experiment III.)

*Monkey 8.*—Confluent eruptions had been produced by inoculation with pustule contents from two Jamaican alastrim patients (L and F) 17 days previously. (Experiment III.)

*Monkey 10.*—Normal control.

Twenty-four hours after inoculation:

*Monkey 1.*—Slight elevation of scratches.

*Monkey 6.*—Elevation of scratches measuring 5 millimeters across.

*Monkey 8.*—Elevation of scratches measuring 8 millimeters across.

*Monkey 10.*—Slight elevation measuring 4 millimeters across.

Forty-eight hours after inoculation:

*Monkey 1.*—Very slight induration along scratches.

*Monkey 6.*—At anterior end of all scratches there are fine papules, both along the scratch and in the adjacent skin. Posterior ends of scratches show slight induration.

*Monkey 8.*—Elevation of scratches has decreased to 3 millimeters in width.

*Monkey 10.*—Slight induration along scratches.

Three days after inoculation:

*Monkey 1.*—Pinkish papules measuring 2 to 3 millimeters numerous on all three scratches and scattered in area between. Some vesiculation beginning.

*Monkey 6.*—The fine papules have become vesicular and show signs of drying in centers. There is a slight areola formation.

*Monkey 8.*—One scratch shows a pinkish papule and one doubtful papule; another shows four pinkish papules, and the third shows no papules.

*Monkey 10.*—An area measuring 3 millimeters on each side of the scratch along its entire length is pinkish and slightly indurated. Overlapping papules are suggested by the scalloped border of this induration.

Four days after inoculation:

*Monkey 1.*—Serocrusting of practically all lesions; those not crusting are pinkish.

*Monkey 6.*—Definite serocrusts with pseudoumbilication; lesions about 5 millimeters in diameter. Monkey has scratched crusts from some of the lesions, exposing bleeding surfaces,

*Monkey 8.*—All lesions have yellowish crusts.

*Monkey 10.*—Slight crusting along scratches; areola deeper pink; papulation further advanced; three accessory papules between scratches.

Five days after inoculation:

*Monkey 1.*—All lesions have crusted; there is still slight pinkish papulation.

*Monkey 6.*—Yellow serocrusts on scratches. Red crusts on lesions where crusts have been scratched off.

*Monkey 8.*—One lesion shows reddening, others have practically disappeared.

*Monkey 10.*—First scratch shows a yellowish-green crust 6 to 7 millimeters across with a pink areola 3 millimeters wide surrounding it; total width of involvement 12 to 13 millimeters. Second scratch, 4 millimeters crust, 2 to 3 millimeters areola surrounding it. Third scratch, 1 to 2 millimeters crust, pinkish areola 2 to 3 millimeters surrounding. Slight umbilication of one accessory papule.

Seven days after inoculation:

*Monkey 1.*—There is a raised area on each scratch measuring 6 millimeters in width surmounted by a crust 4 millimeters in width. The isolated papules between the scratches measure 4.5 millimeters in diameter with a crust 1.5 millimeters in diameter. The crust is brownish, with a narrow raised margin not reddened.

*Monkey 6.*—Marked general pustulation with crusting. No areola. Area of involvement measures 10 millimeters across each scratch. Resembles a tenth-day vaccinia. Some crusts have been scratched off.

*Monkey 8.*—A single crusted pustule; one accessory pustule with crust.

*Monkey 10.*—All lesions are markedly pustular with a surrounding areola 1 millimeter in width. The pustule bands have yellowish crusts in center. An accessory pustule between scratches consists of an elevated slightly red papule measuring 7 millimeters in diameter, surmounted by a vesicle 4.5 millimeters in diameter.

Eight days after inoculation:

*Monkey 1.*—All lesions have brownish crusts; the raised margin has dried and become yellowish.

*Monkey 6.*—The brownish crusts are drying. Where detached the exposed surface has the appearance of raw beef, but shows no pus.

*Monkey 8.*—Crusts practically all detached, exposing a healed linear scar.

*Monkey 10.*—Monkey has scratched tops from some of the lesions, revealing a bleeding surface, similar to fourth-day appearance on monkey 6 Undetached crusts raised to width and thickness of a little finger. Crusts composed of yellow, confluent lesions, circular, with depressed centers. Evidently in *M. cynomolgus* the usual distended vesicles have a tendency to have thick yellow walls and appear more shrunken than in *M. rhesus.* (Monkey 4, Experiment II.) The surface under crusts is bleeding as though congestion was intense.

Nine days after inoculation:

*Monkey 1.*—Crusts on all three scratches range from 2.5 to 7.5 millimeters in width. The crusts on accessory lesions are 2.5 millimeters in diameter. No redness.

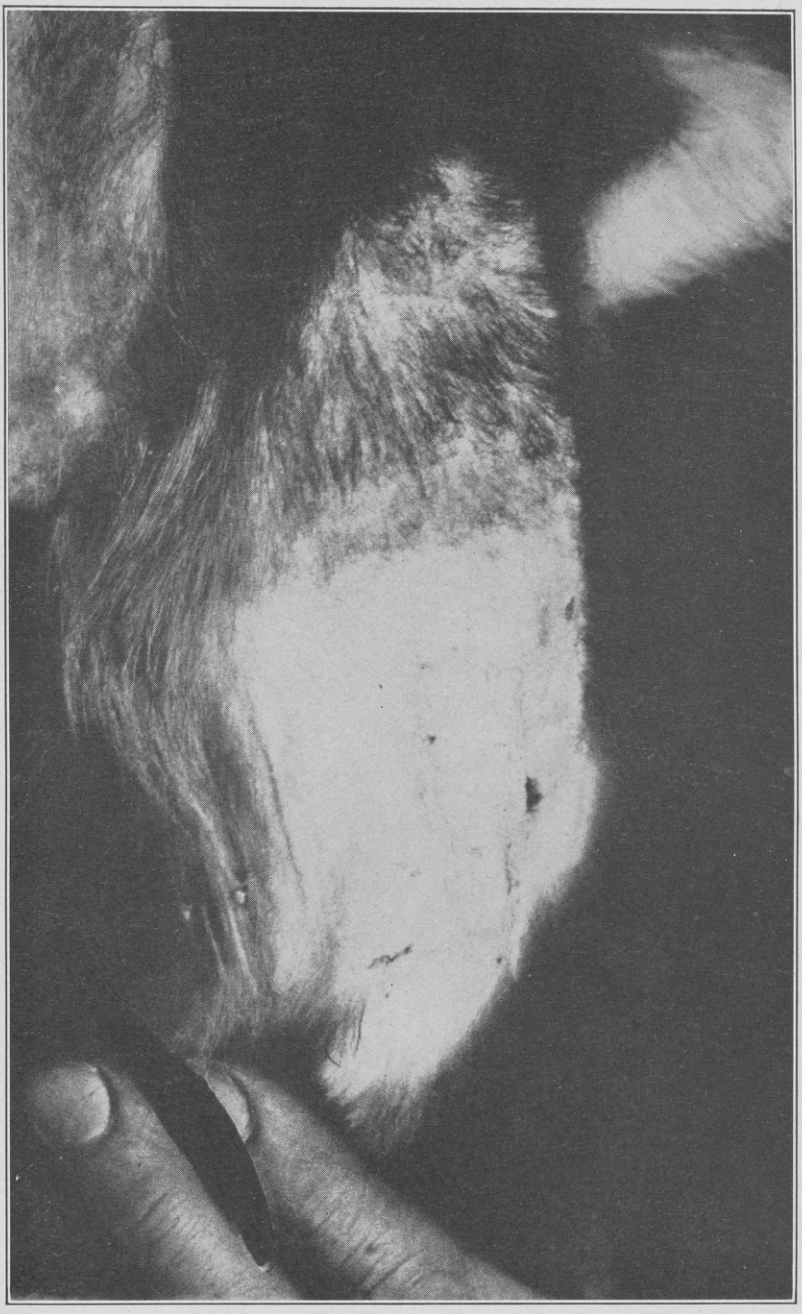

Fig. 22.—Monkey 8, showing immunity to vaccinia following inoculated alastrim. Twenty-six days previously, this monkey had been successfully inoculated with alastrim (see Fig. 15 and Experiment III. Nine days previous to this photograph vaccine virus had been inserted along three linear scratches, the remains of which may be seen in the shaved area. The normal control monkey, vaccinated at the same time, reacted with a typical vaccinia as shown in Fig. 23. (Experiment V)

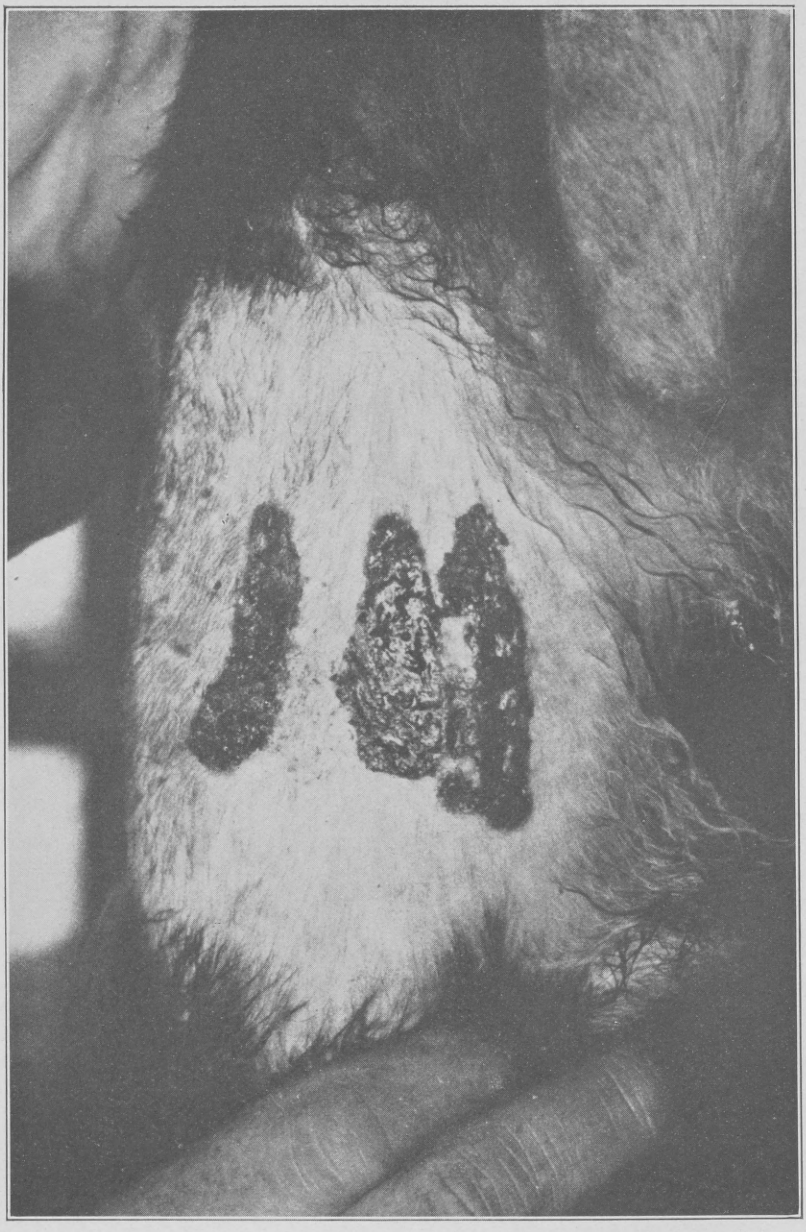

Fig. 23—Monkey 10, nine days after vaccination with a potent vaccine virus. This monkey served as a control for Monkeys 1, 6, and 8. (Fig. 22 and Experiment V)

*Monkey 6.*—Crusts on all three scratches, measuring on the first scratch 8 to 12 millimeters in width, on the second scratch 3 to 3.5 millimeters in width, and on the third scratch 1 to 4.5 millimeters in width. Crusts drier and further advanced than in monkey 10.

*Monkey 8* —Small linear scars. (Fig. 22.)

*Monkey 10.*—Crusts flattened, but bleed easily if rubbed. Areas involved: 10 by 40 millimeters, 15 by 35 millimeters, and 10 by 40 millimeters. (Fig. 23.)

Ten days after inoculation:

*Monkey 1.*—No change. Crusts dried on all scratches.

*Monkey 6.*—Crusts dried on all scratches.

*Monkey 8.*—No change; small scars. Observations concluded.

*Monkey 10.*—Lesions drying.

Eleven days after inoculation:

*Monkey 1.*—Found dead. Osteomalacia without tuberculosis. Vaccine lesions healing; sections show epithelium regenerated except for portion 0.7 to 2.6 millimeters wide at center of two of the scratches.

*Monkey 6.*—No change.

*Monkey 10.*—Dried crusts.

Twelve days after inoculation:

*Monkey 6.*—Crusts beginning to separate; skin healed beneath.

*Monkey 10.*—If crusts are detached, a deep ulcer is exposed.

Fourteen days after inoculation:

*Monkey 6.*—Only small portion of crusts remaining.

*Monkey 10.*—Thin secondary crusts have formed on ulcers where crusts have been detached.

Fifteen days after inoculation:

*Monkey 6.*—Crusts still separating.

*Monkey 10.*—Thin secondary crusts detached.

Seventeen days after inoculation:

*Monkey 6.*—Lesions healed and almost all crusts detached.

*Monkey 10.*—Where secondary crusts are detached, ulcerated surfaces are still present.

*Results.*—A monkey (monkey 1) successfully inoculated 79 days previously with alastrim (18 lesions produced) and unsuccessfully vaccinated 66 days previously, was revaccinated with potent smallpox vaccine. A vaccinoid resulted, reaching its height about the fourth day after vaccination. A monkey (monkey 6) inoculated successfully with smallpox 17 days previously (8 lesions produced) was vaccinated with potent smallpox vaccine. A vaccinoid resulted, reaching its height about the sixth day after vaccination. A monkey (monkey 8) inoculated successfully with alastrim 17 days previously (confluent eruption produced on two sites) was vaccinated with potent smallpox vaccine. A few papules were produced which reached their height on the third day after vaccination and rapidly disappeared (reaction

of immunity). A monkey (monkey 10) used as a control was vaccinated with the same vaccine used on the other monkeys in the experiment. A vaccinia resulted, reaching its height on the eighth or ninth day after vaccination.

A smallpox vaccine which produced typical vaccinia in a normal monkey produced a reaction of immunity in a monkey recently inoculated with alastrim; an early vaccinoid in a monkey inoculated with alastrim two and a half months previously; and a late vaccinoid in a monkey which had recently been inoculated with smallpox but had only eight lesions resulting.

TABLE III.—*Test of immunity to smallpox and alastrim in monkeys previously inoculated with alastrim or smallpox, Experiment VI*

| Monkey No. | Previous inoculations | | | | Test inoculations | | |
|---|---|---|---|---|---|---|---|
| | Strain | Immediate source | Days prior to this test | Result | Strain | Immediate source | Result |
| 2 | Haitian alastrim. | Human crusts. | 84 | Eruption, 8 lesions, covered by crusts until ninth day. | Smallpox, M and N. | Through monkeys 5 and 6, immediately from monkey 9. | Negative, or early reaction second day. |
| | Jamaican alastrim G. | Pustule contents from human patient. | 84 | Eruption, 7 lesions, covered by crust until ninth day. | | | |
| | Jamaican alastrim H. | do | 84 | Negative | Jamaican alastrim L. | Through monkey 8, immediately from monkey 9. | Do. |
| | Jamaican alastrim G. | do | 71 | Negative, or early reaction first observed fifth day; disappeared seventh day. | | | |
| 5 | Smallpox N. | Human crusts. | 22 | Eruption, 3 lesions, beginning seventh day; papules eighth day. | Jamaican alastrim F. | do | Do. |
| | Smallpox M. | do | 22 | Eruption, 2 lesions, beginning tenth day. | Smallpox M-N. | Through monkeys 5 and 6, immediately from monkey 9. | Do. |
| 7 | Jamaican alastrim G. | Crusts from monkey 2. | 22 | Eruption, 1 papule first observed eleventh day. | Jamaican alastrim L. | Through monkey 8, immediately from monkey 9. | Do. |
| | Haitian alastrim. | do | 22 | Negative | Jamaican alastrim F. | do | Do. |
| 11 | None | (Control) | | | Smallpox M and N. | Through monkeys 5 and 6, immediately from monkey 9. | Eruption, 80 lesions, beginning sixth day. |
| | | | | | Jamaican alastrim F. | Through monkey 8, immediately from monkey 9. | Eruption, generally confluent along scratches beginning fifth day. |
| | | | | | Jamaican alastrim L. | do | Do. |

EXPERIMENT VI (Table III)

June 18, 1921: One *Macacus rhesus* monkey and three *Macacus cynomolgus* monkeys were inoculated with crusts collected the same day from monkey 9. (Experiment IV.) The emulsions of crusts were inoculated into scratches made with a Japanese calf scarifier. Cross scarifications were not used.

*Monkey 2.*—Successfully inoculated 84 days previously with Jamaican alastrim G and Haitian alastrim. (Experiment I.) Unsuccessfully reinoculated 13 days later with Jamaican alastrim G. (Experiment II.) Inoculated on the left side anteriorly with crusts collected from an eruption of 60 lesions which was produced on monkey 9 (Experiment IV) by crusts collected from 13 lesions which were produced on monkeys 5 and 6 by crusts from smallpox patients M and N (SPM–N (M5–6 to M9)). Inoculated on left side posteriorly with crusts collected from an eruption 70 per cent confluent which was produced on monkey 9 (Experiment IV) by crusts from a confluent eruption which was produced on monkey 8 by pustule contents from Jamaican patient L (JL (M8 to M9)).

*Monkey 5.*—Five lesions produced by inoculation with smallpox crusts 22 days previously. (Experiment III.) Inoculated on the right side posteriorly with crusts from smallpox eruption on monkey 9 (SPM–N (M5–6 to M9)). Inoculated on the left side posteriorly with crusts collected from an eruption 60 per cent confluent which was produced on monkey 9 (Experiment IV) by crusts from a confluent eruption which was produced on monkey 8 by pustule contents from Jamaican patient F (JF (M8 to M9)).

*Monkey 7.*—One crusted papule produced by inoculation 22 days previously (Experiment III) with crusts from an eruption which was poduced on monkey 2 by pustule contents from Jamaican patient G. Inoculated on right side posteriorly with JF (M8 to M9). Inoculated on left side posteriorly with JL (M8 to M9).

*Monkey 11.*—Normal control. Inoculated on the right side posteriorly with JL (M8 to M9); on the left side posteriorly with JF (M8 to M9); and on the left side anteriorly with SPM–N (M5–6 to M9). Control scarification made on right side anteriorly.

Forty-eight hours after inoculation:

*Monkey 2.*—Induration 1 millimeter wide along line of scratches, yellowish crusting with some sero-pus exuding.

*Monkey 5.*—Slight yellowing in some of the scratches.

*Monkey 7.*—Slight yellowing in scratches.

*Monkey 11.*—Reddened scratches with some blood stains between. Control area shows no effect of rubbing. Area inoculated with smallpox material has thin yellow crust.

Three days after inoculation:

*Monkey 2.*—Yellow crusts have become drier. In some places a brownish crust covers several adjacent scratches.

*Monkey 5.*—Yellow exudate drying.

*Monkey 7.*—Yellow exudate drying.

*Monkey 11.*—No change.

Four days after inoculation:

*Monkey 2.*—Crusts on smallpox area drying and beginning to separate. Some redness in deeper scratches on JL (M8 to M9), crusts partly off.

*Monkey 5.*—Crusts off on smallpox area, linear scars. Yellow crusts on JF (M8 to M9).

*Monkey 7.*—Crusts separating.

*Monkey 11.*—No change on control area. SPM–N (M5–6 to M9): Thick beeswaxlike crust. JL (M8 to M9) and JF (M8 to M9): Crusts between scratches separating. Some reddening around scratches.

Five days after inoculation:

*Monkey 2.*—No change.

*Monkey 5.*—No change.

*Monkey 7.*—No change.

*Monkey 11.*—Smallpox area still shows beeswaxlike crust. Both alastrim areas show confluent red papules approximately 2 millimeters in diameter along lines of scratches.

Six days after inoculation:

*Monkey 2.*—A few inactive tiny papules where crusts have separated.

*Monkey 5.*—No change.

*Monkey 7.*—No change.

*Monkey 11.*—Papules on alastrim areas show depressed centers. Beginning vesiculation and yellow crusting. Papules and a very few vesicles on smallpox area.

Seven days after inoculation:

*Monkey 2.*—No lesions visible.

*Monkey 5.*—Crusts separated.

*Monkey 7.*—Crusts separated.

*Monkey 11.*—Control area shows four tiny papules, each one at the top of a scratch. Papules on smallpox area drying. Craterlike pits where crusts removed. JF (M8 to M9): Vesiculation general. JL (M8 to M9): Crusts drying on surface but bleeding where detached.

Nine days after inoculation:

*Monkeys, 2, 5, and 7.*—Linear scars.

*Monkey 11.*—Control area: Little papules crusted. SPM–N (M5–6 to M9): The individual lesion is a reddish papule 2 millimeters in diameter, surmounted by a crust 1.5 millimeters in diameter. The

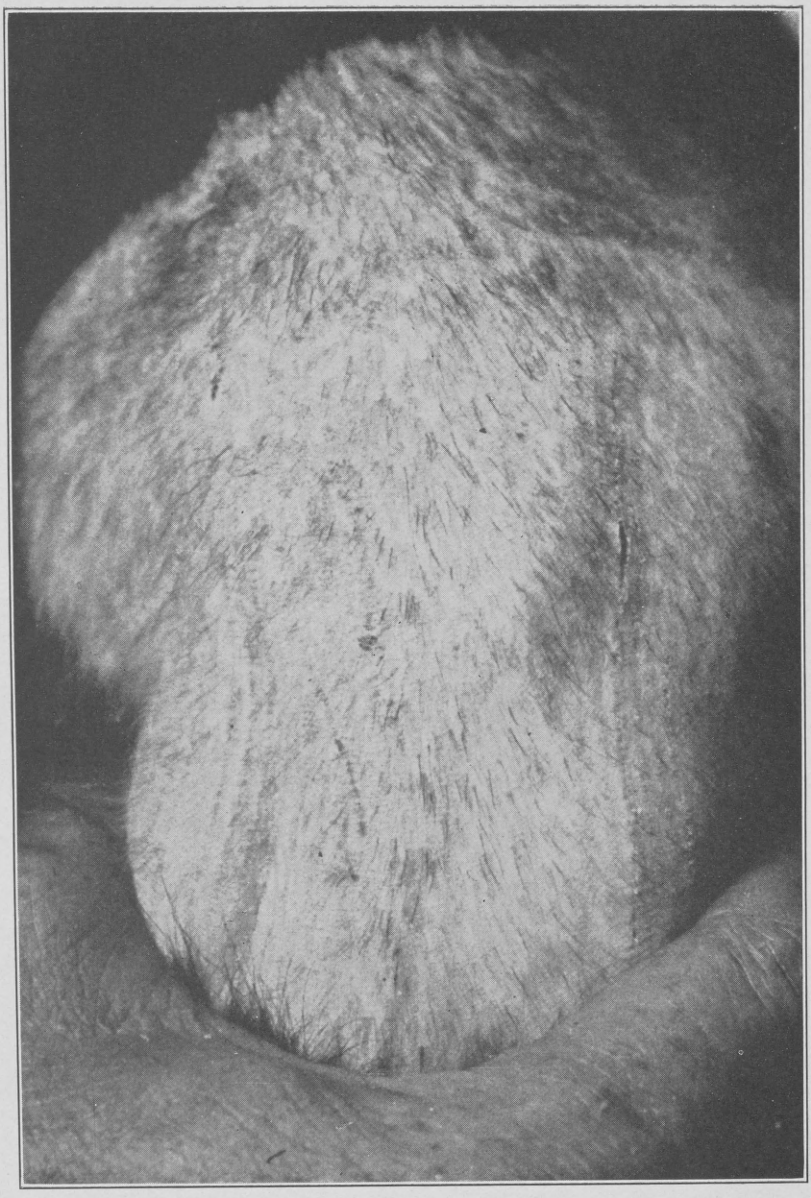

Fig. 24.—Monkey 5, showing immunity to alastrim and smallpox, following inoculated smallpox. Thirty-two days previously this monkey had been inoculated with crusts from smallpox patients, a few lesions resulting. Ten days previous to this photograph smallpox material which had been through two monkeys successively was inoculated by closely parallel linear scratches on the right, and alastrim material (Jamaican F) also after two monkey passages, on the left. There was no noteworthy visible result from the second inoculations except the trauma of the scratches. The nonimmune control monkey inoculated at the same time (Monkey 11) gave highly developed lesions, see Fig. 25. (Experiment VI)

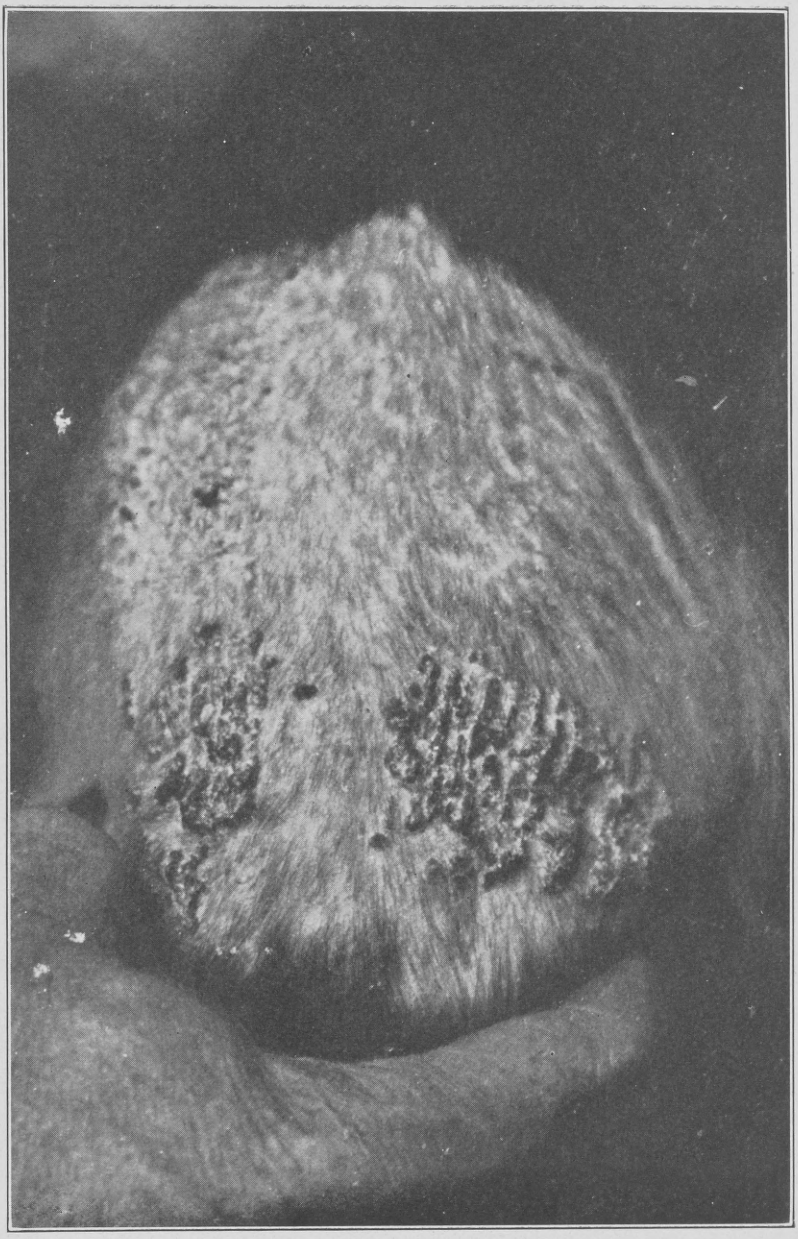

Fig. 25.—Monkey 11, ten days after inoculation with smallpox material (second monkey passage, upper left quadrant), and alastrim material (second monkey passages of Jamaican strains L and F, lower right and left quadrants, respectively). The upper right quadrant was scarified in the same way as the other three quadrants but no material was applied. This production of an eruption on a normal monkey served as a control for the immune monkeys 2, 5 (Fig. 24), and 7 of Experiment VI

crusts are drying and there are pits exposed where crusts have been removed. The eruption is discrete, but covers most of the inoculated area. The two alastrim areas have confluent eruptions along inoculation scratches, with thicker crusts; otherwise similar to above.

Ten days after inoculation:

*Monkeys 2, 5, and 7.*—Linear scars only. Monkey 5 photographed. (Fig. 24.) Observations concluded.

*Monkey 11.*—Crusts scratched off one of confluent areas of smallpox eruption. (Fig. 25.)

Eleven days after inoculation:

*Monkey 11.*—Heavy dark brown crusts on all areas except smallpox, where they are lighter.

Twelve days after inoculation:

*Monkey 11.*—Some crusts are detached from the smallpox area. No change in appearance of crusts.

Fourteen days after inoculation:

*Monkey 11.*—Crusts separating.

*Results.*—A monkey (monkey 2) successfully inoculated 84 days previously with Jamaican and Haitian alastrim, and unsuccessfully reinoculated 13 days later with Jamaican alastrim, was inoculated without result with smallpox material which had been passed through two monkeys successively. The same monkey was inoculated with alastrim material (originally from patient L) which had been passed through two monkeys successively. A few tiny papules of a transient character resulted. A monkey (monkey 5) successfully inoculated 22 days previously with smallpox crusts (eight lesions produced) was inoculated with the above smallpox material. The same monkey was inoculated with alastrim material (originally from patient F) which had been passed through two monkeys successively. Only linear scars marking the scarifications were produced on either site. A monkey (monkey 7) on which had been produced one lesion by inoculation, 22 days previously, with crusts from an eruption which was produced on monkey 2 by pustule contents from patient G, was inoculated with the above two strains of alastrim material used on monkeys 2 and 5. Linear scars resulted from the scarification; no other lesions were produced. A normal monkey was inoculated with the above smallpox and alastrim materials as a control to monkeys 2, 5, and 7. Characteristic eruptions were produced on all inoculation sites. Alastrim material (second monkey passage) and smallpox material (second monkey passage) capable of producing eruptions in a normal monkey (third monkey passage) failed to produce eruptions in monkeys previously immunized by alastrim material (first monkey passage), alastrim material (second monkey passage), and smallpox material (first monkey passage).

TABLE IV.—*Test of immunity to smallpox and alastrim in monkey previously vaccinated with vaccinia, Experiment VII*

| Monkey No. | Previous inoculation | | | Test inoculations | | |
|---|---|---|---|---|---|---|
| | Material | Days prior to this test | Result | Strain | Immediate source | Result |
| 10 | Vaccine virus lot 587. | 10 | Confluent vaccinia, beginning third day, pustules fifth day. | Jamaican alastrim F. | Through monkey 8, then through monkey 9. | Negative. |
| | | | | Jamaican alastrim L. | -----do----- | Do. |
| | | | | Smallpox M and N. | Through monkeys 5 and 6, then through monkey 9. | Do. |
| 12 | None | | (Control) | Jamaican alastrim L. | Through monkey 8, then through monkey 9. | Generally confluent along scratches beginning fourth day. |
| | | | | Jamaican alastrim F. | -----do----- | Do. |
| | | | | Smallpox M and N. | Through monkeys 5 and 6, then through monkey 9. | Eruption, 42 lesions, beginning fourth day papules fifth day. |

**EXPERIMENT VII (Table IV)**

June 23, 1921: Two *Macacus cynomolgus* monkeys were inoculated with crusts collected five days previously from monkey 9. (Experiment IV.) The emulsions of crusts were inoculated into scratches made with a Japanese scarifier. Cross scarifications were not made.

*Monkey 10.*—Successfully inoculated with vaccinia 10 days previously. (Experiment V.) Inoculated on the right side anteriorly with JL (M8 to M9); on the left side anteriorly with JF (M8 to M9); on the left side posteriorly with SPM–N (M5–6 to M9). The lesions of the previous vaccinia occupied the posterior right side.

*Monkey 12.*—Normal control. Inoculated on the left side anteriorly with JL (M8 to M9); on the left side posteriorly with JF (M8 to M9); and on the right side posteriorly with SPM–N (M5–6 to M9). A control scarification was made on the anterior right side.

Twenty-four hours after inoculation:

*Monkey 10.*—Serum crusts along line of scratches, and dried blood.

*Monkey 12.*—Same as monkey 10.

Forty-eight hours after inoculation:

*Monkey 10.*—Serum crusts confluent.

*Monkey 12.*—Serum crusts along line of scratches.

Four days after inoculation:

*Monkey 10.*—No activity on smallpox or alastrim inoculation sites.

*Monkey 12.*—Beginning eruption of slightly pink, definitely elevated 2-millimeter papules on alastrim sites. Slight pinkness on smallpox site.

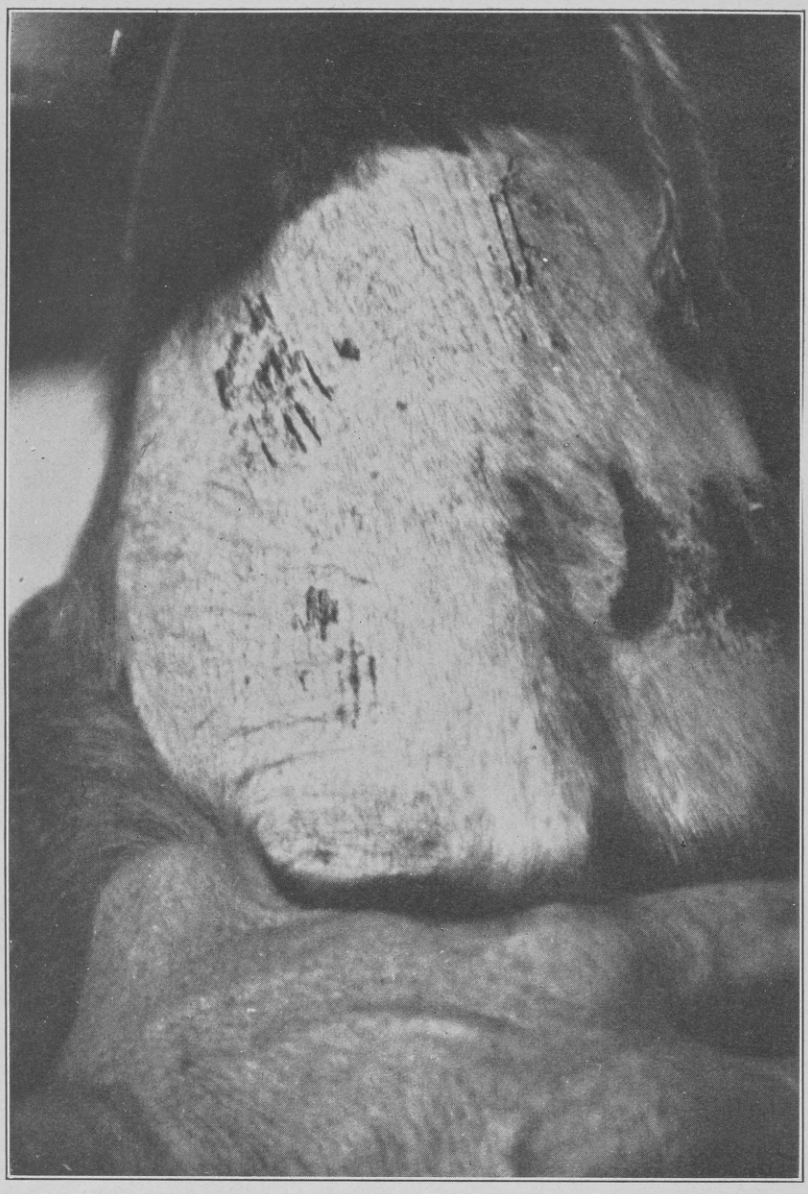

Fig. 26.—Monkey 10, showing immunity to alastrim and smallpox, following vaccinia. Fifteen days previously this monkey had been vaccinated and the resulting lesions may be seen in the lower right quadrant of the monkey's back. Fig. 23 shows the same lesions on the ninth day. Five days before this photograph was taken, the monkey was inoculated with alastrim material (second monkey passage of Jamaican strains L and F, upper right and left quadrants, respectively), and with smallpox material (second monkey passage, lower left quadrant). Only the traumatic lesions resulted. The control monkey (Fig. 27) on the other hand, developed papules, umbilicated vesicles, and crusts, beginning four days after inoculation. (Experiment VII)

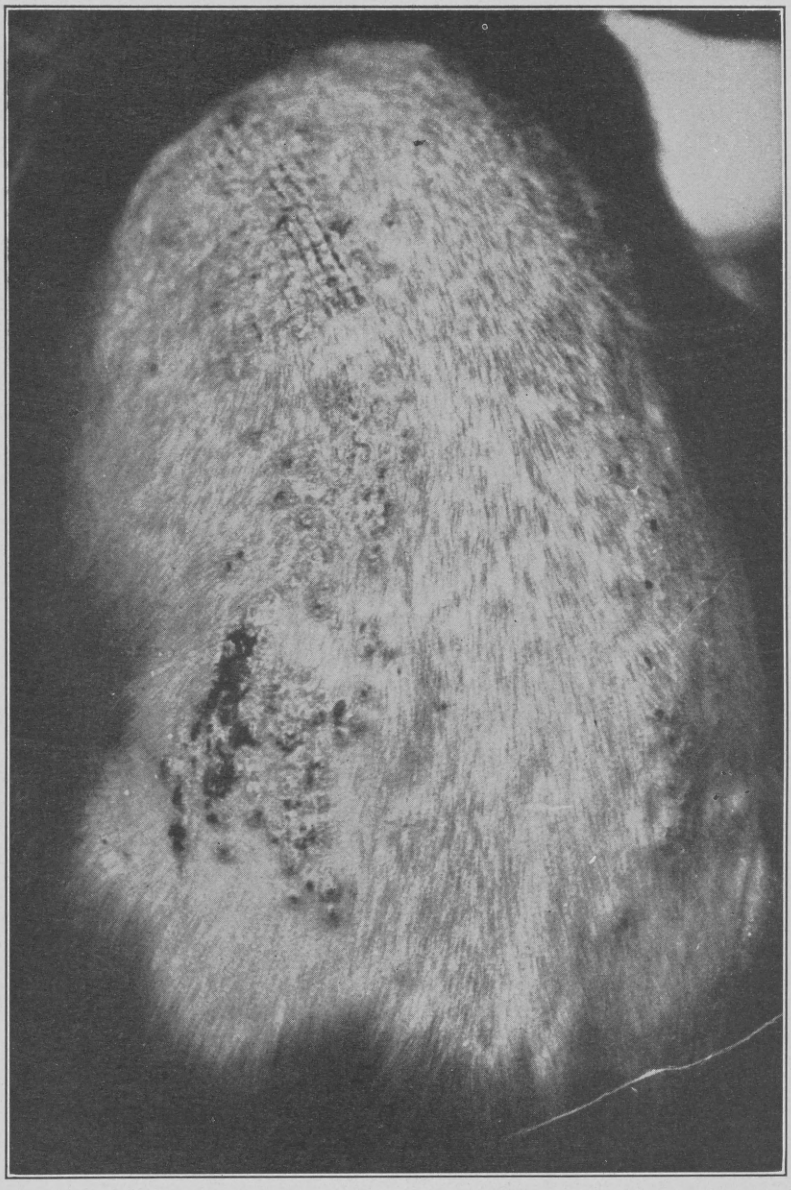

Fig. 27.—Monkey 12, five days after inoculation with alastrim material, second monkey passage (upper and lower left quadrants of back, Jamaican strains L and F), and smallpox material, second monkey passage (right lower quadrant). The beginning vesiculation and umbilication may be seen in some of the more isolated lesions of the left lower quadrant. The right upper quadrant, scarified in the same way, but with no material applied, presents no lesion. This normal monkey served as a control for Monkey 10 (Fig. 26) in Experiment VII

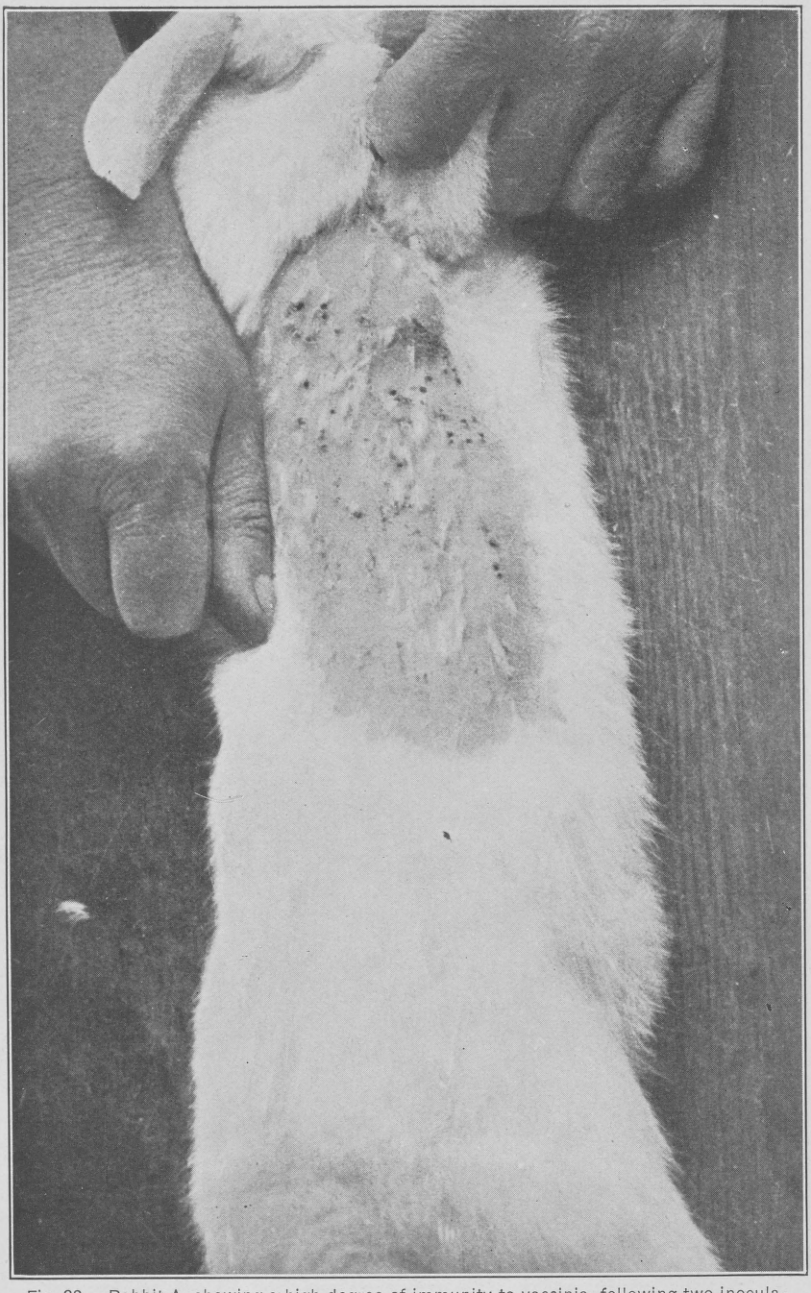

Fig. 28.—Rabbit A, showing a high degree of immunity to vaccinia, following two inoculations with alastrim material 25 and 16 days previously, neither of which produced a definite eruption. Seven days before the photograph was taken, the rabbit was vaccinated over four areas on the right side with a potent vaccine virus using the dilutions 1:1,000, 1:3,000, 1:10,000, and 1:30,000. The number of lesions produced on these areas were 21, 19, 5 and 1, respectively, and the lesions were definitely accelerated. The control rabbit (Fig. 29) showed a confluent eruption over the greater part of the corresponding areas. (Experiment VIII)

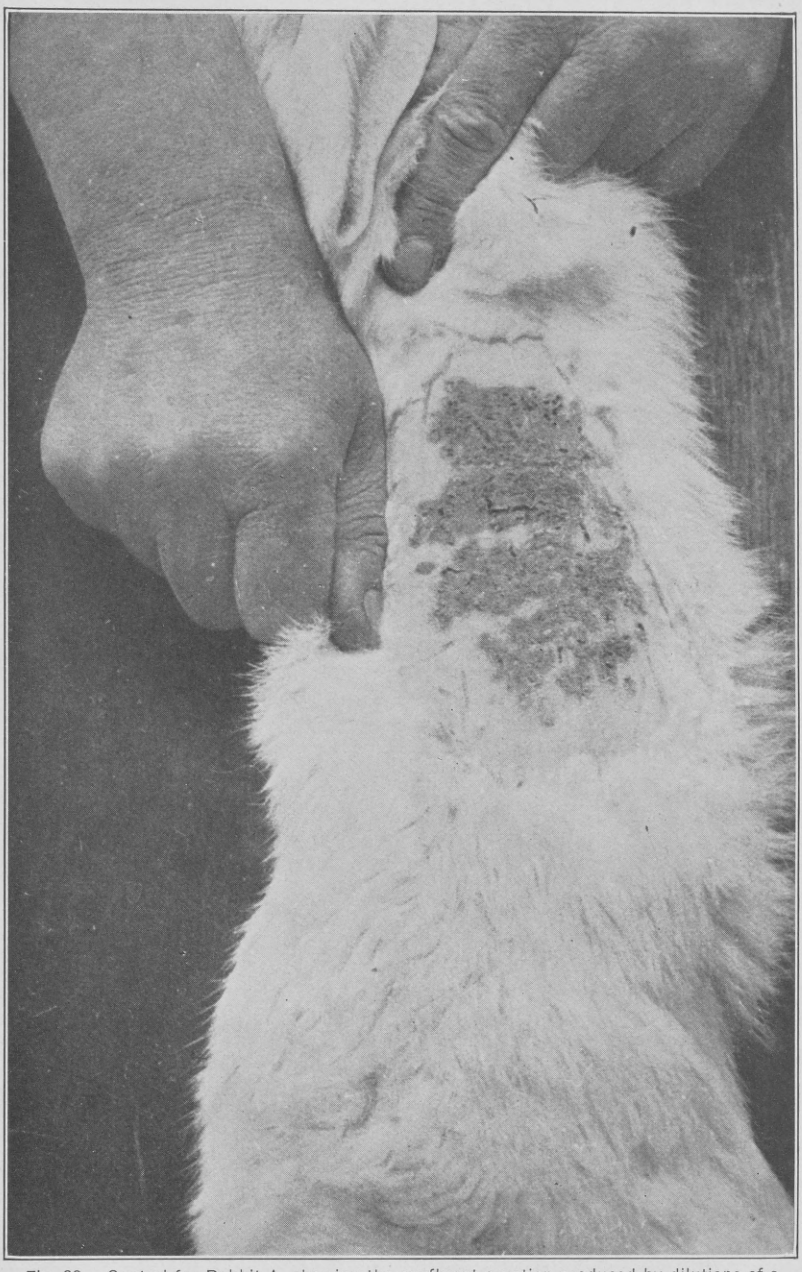

Fig. 29.—Control for Rabbit A, showing the confluent eruption produced by dilutions of a potent vaccine virus on a normal rabbit on the seventh day after vaccination. This rabbit was inoculated with the same dilutions of the same virus as were used to vaccinate Rabbit A, and responded with confluent eruptions covering 100 per cent, 95 per cent, 95 per cent, and 50 per cent of the four areas, respectively. In marked contrast to Rabbit A (Fig. 28) which presented only isolated and accelerated lesions. (Experiment VIII)

Five days after inoculation:

*Monkey 10.*—No change on smallpox or alastrim areas. (Fig 26.) Observations concluded.

*Monkey 12.*—Numerous salmon-colored papules on smallpox site. Papules measure 2.5 millimeters in diameter. Confluent red papules measuring 2 millimeters in diameter on both alastrim sites. Some of these are suggestive of vesiculation. (Fig. 27.)

Six days after inoculation:

*Monkey 12.*—Beginning vesiculation on smallpox areas, more advanced on alastrim areas.

Seven days after inoculation:

*Monkey 12.*—On smallpox area, 42 discrete lesions, some vesicular, some beginning to crust. On both alastrim areas, a confluent eruption of somewhat crusted vesicles. Isolated lesions markedly elevated and well distended. Monkey has begun to scratch lesions.

Nine days after inoculation:

*Monkey 12.*—Brownish-yellow crusts formed on all lesions.

*Results.*—Alastrim material (second monkey passage) and smallpox material (second monkey passage) capable of producing typical eruptions in a normal monkey failed to produce eruptions in a monkey inoculated with smallpox vaccine 10 days previously.

EXPERIMENT VIII

March 28, 1921: Rabbit A was shaved and inoculated with alastrim material (Jamaican G), 1 in 10 suspension on the right side, 1 in 50 on the left side. The technique was like that described for the inoculation of the monkeys with alastrim. Rabbit B was similarly inoculated with Jamaican H.

April 6 1921: During the nine days following inoculation no eruption had appeared at any of the inoculated sites. Rabbit A was reinoculated with scrapings from the eruption produced by the Haitian crusts on monkey 1. Rabbit B was reinoculated with scrapings from the eruption produced by Jamaican G pustule contents on monkey 1. Three days after reinoculation a slight patchy erythema appeared on rabbit A, which disappeared the next day. There was no change in the inoculated area on the other rabbit. The abrasions produced by the inoculation healed quickly.

April 15, 1921: Rabbit A was vaccinated with dilutions of a potent vaccine virus, a normal rabbit being used as a control. The area vaccinated with each dilution measured 2.5 by 5 centimeters. At the end of seven days a few accelerated lesions had developed and dried to tiny brown crusts. (Fig. 28.) The control rabbit had typical seventh-day full vaccinia vesicles. (Fig. 29.)

*Rabbit A and control*

| Dilution of vaccine virus | Eruption on rabbit A | Eruption on normal rabbit |
|---|---|---|
| 1:1,000 | 21 discrete lesions | Confluent, covering 100 per cent of the inoculated area. |
| 1:3,000 | 19 discrete lesions | 95 per cent confluent. |
| 1:10,000 | 5 discrete lesions | Do. |
| 1:30,000 | 1 lesion | 50 per cent confluent. |

April 16, 1921: Rabbit B was vaccinated, using the same technique, virus, and dilutions as rabbit A. A control was also vaccinated. The results seven days later were as follows:

*Rabbit B and control*

| Dilution of vaccine virus | Eruption on rabbit B | Eruption on normal rabbit |
|---|---|---|
| 1:1,000 | 6 discrete lesions | Confluent, covering 75 per cent of the inoculated area. |
| 1:3,000 | 3 discrete lesions | 50 per cent confluent. |
| 1:10,000 | No lesions | 40 per cent confluent. |
| 1:30,000 | ----do-- | 5 discrete lesions. |

Twenty other normal rabbits have been inoculated before and since with dilutions of this vaccine virus, using the same technique, and in none has the eruption been so accelerated or so scanty as that observed on rabbits A and B.

*Results.*—A rabbit (A) was inoculated cutaneously with alastrim pustule contents (Jamaican G), which had produced eruptions on two monkeys. No eruption was produced on the rabbit. Nine days later the rabbit was reinoculated cutaneously with scrapings from an eruption produced in a monkey by Haitian alastrim. A transient erythema was produced. Nine days after the reinoculation the rabbit was again inoculated cutaneously with dilutions of a potent vaccine virus and showed a few tiny scattered lesions which were definitely accelerated and had become crusted on the seventh day, at which time a confluent eruption on a control rabbit was at its height. The rabbit was, therefore, almost completely immune to vaccine virus.

Another rabbit (B) was inoculated with alastrim pustule contents (Jamaican H) which produced no results on monkeys. No eruption was produced on this rabbit. Nine days later the rabbit was reinoculated with scrapings from an eruption produced on a monkey by Jamaican G alastrim. No eruption was produced. Ten days after the reinoculation, the rabbit was vaccinated with essentially the same results as in rabbit A.

Two rabbits were therefore immunized to alastrim to such a degree that, though they showed no eruption, they were later observed to be

almost completely immune to vaccine virus, the scanty lesions being in no sense imperfect in development but definitely accelerated, as in vaccinoid.

EXPERIMENT IX

June 18, 1921: Six albino rabbits were shaved on one side on June 17 and inoculated with emulsions of crusts collected this day from monkey 9. The inoculations were done with blunt pipettes into scarifications made by a Japanese scarifier.

*Rabbits E and F.*—SPM–N (M5–6 to M9).

*Rabbits G and H.*—Anteriorly with JL (M8 to M9); posteriorly with JF (M8 to M9).

*Rabbits I and K.*—JG (M2 to M7–8 to M9). Crusts, the source of which was alastrim material (pustule contents) from Jamaican patient G, which material had been passed through monkey 2 to monkeys 7 and 8, collected from these monkeys, mixed and inoculated on monkey 9.

Forty-eight hours after inoculation:

All rabbits show red crusts from effects of rubbing.

Three days after inoculation:

Color fading from red crusts, leaving serum crusts on surface.

Five days after inoculation:

*Rabbit E.*—Crusts desquamating; a few doubtful papules.

*Rabbit F.*—No papules.

*Rabbit G.*—Maculo-papules on both areas.

*Rabbit H.*—A few maculo-papules.

*Rabbit I.*—No papules.

*Rabbit K.*—One or two bleeding points where crusts are detached; no papules.

Six days after inoculation:

*Rabbit E.*—A few discrete papules, light red, 2 to 4 millimeters in diameter.

*Rabbit F.*—Negative.

*Rabbit G.*—No change.

*Rabbit H.*—Papules increased in size.

*Rabbit I.*—Negative.

*Rabbit K.*—Negative.

Seven days after inoculation:

*Rabbit E.*—Papules redder and more distinct.

*Rabbit F.*—Negative.

*Rabbit G.*—A few maculo-papules above scarified area as well as within it.

*Rabbit H.*—A few definite papules, some outside the sacrificed area.

*Rabbit I.*—No change.
*Rabbit K.*—No change.
Nine days after inoculation:
*Rabbit E.*—Papules still present.
*Rabbit F.*—Negative.
*Rabbit G.*—Definite papules.
*Rabbit H.*—A few of the papules have glistening tops.
*Rabbit I.*—Negative.
*Rabbit K.*—Negative.
Ten days after inoculation:
*Rabbit E.*—Papules receding.
*Rabbit F.*—Negative.
*Rabbit G.*—Papules receding.
*Rabbit H.*—Papules receding.
*Rabbit I.*—Negative.
*Rabbit K.*—Negative.

*Results of first inoculation.*—Smallpox material passed successively through two monkeys, produced a few papules on one of two rabbits (rabbit E) and no visible result on another rabbit (rabbit F). Alastrim material passed successively through two monkeys produced a few papules on two rabbits (rabbits G and H). Alastrim material from another case passed successively through three monkeys produced no apparent result on two rabbits (rabbits I and K).

June 28, 1921: The above-mentioned six rabbits, E to K, inclusive, together with a control rabbit (rabbit L), were shaved and vaccinated with a potent smallpox vaccine on the side which had not been shaved June 17. Scarifications were made with a Japanese scarifier and a 1:1,000 dilution of the vaccine was rubbed in by means of blunt pipettes. An area approximately 100 by 50 millimeters was vaccinated.

Four days after inoculation:

*Rabbit E.*—Semiconfluent eruption with yellow serum crusts overlying. Bleeding if serum crusts detached. Vesicles under crusts much smaller than in a seventh-day vaccinia. Otherwise resembles height of the process.

*Rabbit F.*—Resembles rabbit E.

*Rabbit G.*—A discrete eruption of papules with tiny brown crusts, lesions approximately 1 millimeter in diameter. Somewhat congested where serum crusts detached but resembles an eighth-day vaccinia except in size and scarcity of the lesions.

*Rabbit H.*—Resembles rabbit G. Lesions with brown scabs slightly depressed, when serum crust is removed.

*Rabbit I.*—Patches of yellow serum crust, not as general as in other rabbits, no signs of detachment, very slight congestion, no eruption.

*Rabbit K.*—Yellow serum crust over entire area. When detached reveals discrete eruption, some lesions congested, some tiny brown crusts. Resembles rabbits E and F.

*Rabbit L.*—Control. A papular plaque over the entire area, not much serum crust, beginning vesicles along scratches of inoculation.

Six days after inoculation:

*Rabbit E.*—Eighty-six discrete lesions, showing crusting. When crusts were detached some lesions were healed and some were oozing.

*Rabbit F.*—Serum crusts still present. When removed, pits are revealed, some healed, some oozing if crust is detached. No bloody scabs formed.

*Rabbit G.*—Resembles rabbit E. Discrete eruption, shallow pits. Slight oozing in some places where crusts detached. Other lesions healed.

*Rabbit H.*—Almost all lesions healed.

*Rabbit I.*—Found dead yesterday. Cadaver observed this day. On scraping off serum crusts, discrete brownish freckle-like macules were revealed.

*Rabbit K.*—Patchy serum crusts, with brownish crusts on lesions. When brownish crusts, are removed, pits are revealed, mostly healed; eruption discrete, some oozing.

*Rabbit L.*—Control. Brownish crusting of entire surface. Wherever removed, patchy confluent bleeding lesions exposed. No signs of healing.

Seven days after inoculation:

*Rabbit E.*—Thin crusts where serum crusts were detached yesterday. Edges of brownish crusts separating from individual lesions.

*Rabbit F.*—Thin secondary crusts, where detached yesterday. Other lesions healing.

*Rabbit G.*—Thin secondary crusts where detached yesterday. Small original crusts beginning to separate as in rabbit E.

*Rabbit H.*—All lesions healed.

*Rabbit K.*—Thin secondary crusts where detached yesterday. Other lesions healed.

*Rabbit L.*—Control. Crusts were detached yesterday, thicker and heavier than on other rabbits. No healed area. Heavy crust where not detached yesterday.

*Results of second inoculation.*—Based on the degree of healing as compared with the control animal, as well as on the number and acceleration of lesions appearing in the vaccinated area, the order of immunity to vaccinia of these rabbits (exclusive of rabbit I) would appear to be as follows: Rabbits G and H, rabbit K, rabbits E and F. The lesions, however, were accelerated in all instances.

This shows that the alastrim material used, originally from pustule contents, produced a higher degree of immunity than the material derived from smallpox crusts. In general, this has been found to be true in each of the experiments.

Smallpox vaccine in a dilution of 1:1,000, capable of producing a confluent eruption of typical vaccinia on a normal rabbit, produced a discrete eruption of accelerated lesions on rabbits inoculated 10 days previously with crusts from smallpox and alastrim sources.

## DISCUSSION

There are certain objections which may be made to the drawing of broad conclusions from these experiments, but it is believed that these objections are outweighed by the character and variety of the evidence.

Of the nine possible immunization tests which can be made with the three viruses of alastrim, smallpox, and vaccinia all but one have been tried, vaccinia versus vaccinia. It may be objected that in many of these tests only one animal was used. On the other hand, the tests resulted so uniformly in showing immunity that a definite immunological relationship can be predicated. Multiplication of the animals on test so as to determine whether there might be a percentage of failure, while desirable, is hardly necessary. It has been pointed out that other experimenters, such as Greene, who used a larger number of monkeys, did not record their detailed daily observations so that the early appearance and early subsidence of the immune reactions can not be followed, and positive immunity in an animal might actually be recorded as absence of immunity. (See Experiment V.)

Histological study of the lesions was omitted. It was felt that the time element in the appearance of the lesions and their gross development was more characteristic than microscopic changes. The possibility of the lesions produced being nonspecific pyogenic skin infections and the resulting immunity being simply a raising of resistance to pyogens, is not considered likely on account of the slow development of the lesions; it was not until from four to seven days after inoculation that the eruption of alastrim appeared. (See Table V.)

TABLE V.—*Transmission of various strains of alastrim, smallpox, and vaccine virus through monkeys*

| Monkey | Haitian alastrim | | Jamaican alastrim patients | | | | | | | | Smallpox patients | | | | Vaccine virus | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | G | | H | | L | | F | | N, W. Va. | | M, D. C. | | | |
| | Les. | Day | Les. | Day | Les. | Day | Les. | Day | Les. | Day | Les. | Day | Les. | Day | Les. | Day |
| 1 | 5 | <9 | 13 | <9 | 0 | | | | | | | | | | | |
| 2 | 8 | <9 | 7 | <9 | 0 | | | | | | | | | | | |
| 4 | | | | | | | | | | | | | | | Conf. | 4 |
| 5 | | | | | | | | | | | 3 | 7 | 2 | 10 | | |
| 6 | | | | | | | | | | | 8 | 7 | 3 | 10 | | |
| 7 | 0 | | 1 (from Mk. 2) | <11 | | | | | | | | | | | | |
| 8 | 0 (from Mk. 2) | | 2 (from Mk. 2) | <11 | | | Conf. | 7 | Conf. | 7 | | | | | | |
| | | | 0 (weakened virus) | | | | | | | | | | | | | |
| 9 | | | 19 (from Mks. 7 and 8) | 6 | | | Conf. (from Mk. 8) | 5 | Conf. (from Mk. 8) | 5 | 60 (from Mks. 5 and 6) | 5 | | | | |
| 10 | | | | | | | | | | | | | | | Conf. | 3 |
| 11 | | | | | | | Conf. (from Mk. 9) | 5 | Conf. (from Mk. 9) | 5 | 80 (from Mk. 9) | 6 | | | | |
| 12 | | | | | | | Conf. (from Mk. 9) | 4 | Conf. (from Mk. 9) | 4 | 42 (from Mk. 9) | 4 | | | | |

Les.: Number of lesions produced.
Day: Day after inoculation on which eruption first appeared.
< This sign before a number indicates that the eruption developed under a crust and was not discovered until the day indicated.
Conf.: Confluent eruption.
Mk.: Monkey.

The materials used for the first passages into monkeys in the case of Haitian alastrim and of American smallpox were crusts from the human patients; in the case of the four strains of Jamacian alastrim, pustule contents were used which had been phenolized and sealed in glass until the first inoculation.

The inoculations were made on separate areas, but for each monkey the inoculations covered by this table were made simultaneously; the subsequent inoculations for immunity tests are described in Tables I, II, III, and IV.

In Experiment II there was no simultaneous control for monkey 2, inoculated the second time with alastrim, and the material used for this reinoculation, though infectious 13 days previously, was found to be inert 11 days later (monkey 8). This constitutes a valid objection to this test, but the test (alastrim versus alastrim) was repeated on monkeys 2 and 7 with a control, in Experiment VI.

A more important objection is that the inoculations in some of the monkeys were multiple and heterogeneous. This was done for two reasons—in order to compare the different viruses, strains, and generations under the same conditions on the same animal and in order to secure the maximum information from a limited number of animals. It will be noted that no animal which was inoculated with different viruses was subsequently used for immunization tests; also that if viruses collected from such an animal were used, the result on the test animal was negative, so that no eruption on such a test animal could be attributed to one virus when actually due to another by reason of mixture on the propagating monkey. Furthermore, on the two control animals (monkeys 11 and 12) where viruses were used which had been propagated on separate areas of the same monkey, control areas were scarified, using the same technique as on the inoculated areas, and no eruption developed. Monkeys 1, 2, 7, and 8 were also inoculated in part with inert viruses, and there was no evidence of the transfer of any of the active viruses to these areas. It is believed that this confirms the impression that the inoculated disease of alastrim or smallpox is sufficiently difficult to transmit on monkeys so that there is no practical danger of virus from one area being implanted in another by accident. It appeared to require a considerable quantity of virus and prolonged rubbing to secure good eruptions. Fortunately, also, the eruption in the areas inoculated with smallpox was in general somewhat slower in appearance and somewhat more scanty than in those inoculated with the strains of alastrim which were used for the immunity tests in question; thus again one may be reasonably sure that the material from the alastrim areas was alastrim and not smallpox material transferred by accident from the smallpox area. Finally, even if it is granted that such a mixture on the previous propagating animal (monkey 9) took place, the conclusion that there is cross immunity between alastrim and smallpox is not invalidated, for both monkey 5 (previously inoculated with smallpox) and monkey 7 (previously inoculated with alastrim) were immune to all material applied. If this material were predominantly smallpox material, it is proven that alastrim did immunize against smallpox (monkey 7); if the mixture were predominantly alastrim material, it is proven that smallpox immunized against alastrim (monkey 5).

## SUMMARY

A review of the literature indicates the identity of the variolalike disease having a low mortality which has prevailed in the West Indies, Brazil, Africa, and Australia under various names with the mild smallpox of the United States and Canada.

Experiments undertaken to investigate the immunological relationship of the West Indian disease with that of the United States resulted as follows:

A vesico-papular eruption was regularly produced in monkeys, through three generations, by inoculation with material the sources of which were pustule contents from three Jamaican patients with alastrim. Crusts from a Haitian patient with the same disease produced a similar eruption in two monkeys. Crusts from two American patients with mild smallpox (District of Columbia and West Virginia) produced similar eruptions in monkeys through three generations.

Two monkeys successfully inoculated with alastrim were completely immune to vaccine virus 13 and 17 days later; the first of these was partially immune (gave a modified reaction or vaccinoid) to vaccine virus 79 days after the alastrim inoculation.

A monkey successfully vaccinated with vaccine virus was completely immune 10 days later to alastrim and to smallpox inoculation.

A monkey successfully inoculated with smallpox was completely immune 22 days later to alastrim and to smallpox.

Another monkey successfully inoculated with smallpox was partially immune (gave a modified reaction or vaccinoid) to vaccine virus 17 days later.

Two monkeys successfully inoculated with alastrim were completely immune to another inoculation of alastrim 13 and 22 days later, respectively, and the first of these was completely immune to a third inoculation of alastrim and an inoculation with smallpox 84 days after the first inoculation.

Rabbits inoculated with alastrim showed some immunity to vaccine virus; those receiving two inoculations (directly with human material and with the first monkey generation) showed a higher degree of immunity than those inoculated once (with the second or third monkey generation). This immunity was not different from that shown by rabbits which had been inoculated with smallpox (second monkey generation). None of these rabbits showed marked lesions from their smallpox or alastrim inoculations.

The fact that definite cross immunity exists between alastrim and mild smallpox and between alastrim and vaccine virus is additional evidence of the identity of alastrim and mild smallpox.

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