

## THE CUTANEOUS TRICHOPHYTIN REACTION.<sup>1</sup>

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### PLATE XXXIX.

Until recently the trichophyton and allied infections have been regarded as strictly localized diseases without causing any general changes in the organism. The investigations of Calderone (1), Truffi (2), Plato (3), Pautrier and Lutembacher (4) have shown that in cases of trichophytia profunda the infection leads to changes in the organism aside from the local skin lesions. Patients suffering with trichophytia profunda respond to subcutaneous injections of culture filtrates of different strains of trichophyton, called trichophytin, with local and general reaction. The local reaction occurring on the point of injection gives rise to inflammation and infiltration. Besides this reaction on the point of injection, but less constantly and less pronounced, there occurs another local reaction on the diseased area. The general reaction consists of malaise, headache, muscular pains, rise of temperature, etc. Superficial cases of trichophytia do not give any reaction under the usual conditions. The analogy of this reaction with the tuberculin reaction in cases of tuberculosis is very striking and, indeed, it goes even further, as will be shown later. The trichophytin reaction is just as specific as the tuberculin reaction in the sense that it is not observed in healthy individuals or in patients suffering with diseases not due to an infection with hyphomycetes belonging to the trichophyton group. It is of interest that trichophytins prepared from different types of trichophyton derived from clinically different cases act alike, so that this reaction cannot be utilized for the differentiation of various trichophyton infections. This result is the less surprising, as the investigations of Král (5) and Waelsch

<sup>1</sup> Received for publication April 29, 1910.

(6), showing a rather extensive pleomorphism of pathogenic hyphomycetes, taught us to be cautious in basing the classification of these fungi on morphological and cultural differences.

Several investigators tried to produce specific lesions in animals by intravenous subcutaneous and intraperitoneal injections of trichophyton cultures, but without success. Nevertheless, it seems that such injections may produce a certain change in the reactivity of the animals. For instance, Plato injected a trichophyton culture subcutaneously in a rabbit and this animal reacted to an injection of trichophytin given four days later with a definite rise of temperature. The experiments of Citron (7) conducted with the view of producing an active or passive immunity by treating rabbits with trichophyton cultures gave practically negative results. Only a moderate formation of precipitins was found, and again the serum of these rabbits did not only react with the trichophyton used for the immunization, but also with other types.

The investigations of Bloch (8) and Bloch and Massini (9) contributed greatly to the advancement of our knowledge with regard to the altered reactivity of an organism having passed through a trichophyton infection. In his animal experiments he was able to show that a guinea pig having passed through an infection with the culture of a certain trichophyton became immune to a subsequent infection. The unsuccessful attempts of previous investigators to produce immunity by means of intravenous, intraperitoneal and subcutaneous injection prompted him to adopt a different method. The cultures were directly applied to the skin and after a definite period of incubation the disease developed, reached its acme at a definite time and healed spontaneously. The course of the disease is typical and shows a definite division in different periods. A second inoculation given after the spontaneous restitution did not lead to any definite lesions, and it was shown that the immunity was not only limited to that part of the skin which had been the seat of the previous infection, but the whole skin proved refractory to a second inoculation. The intraperitoneal injection of the same culture which on cutaneous inoculation produced immunity was, at the most, capable of diminishing the intensity of a renewed cutaneous infection. It was, furthermore, not possible to obtain an active immuni-

zation of animals by means of "presssaft" or culture filtrates, nor was it possible to immunize animals passively with the serum of immunized animals. But with repeated cutaneous inoculations the time at which the immunity appears could be determined. At intervals of two days inoculations were made around a primary infection. The second and third inoculations, made on the second and fourth days after the primary, were still positive, the fourth, made six days after the primary, led only to reddening and infiltration, while from the seventh day on the inoculations were negative. The time from which the inoculations became negative coincided with the beginning of the spontaneous involution of the first inoculation. The processes, therefore, leading to the spontaneous involution are the same which are responsible for the immunity. These experiments show an unmistakable analogy to those of v. Pirquet with vaccinia. The analogy would be still more striking if it could be shown that the subsequent inoculations in the experiments of Bloch just cited were really not entirely negative, but gave rise to an "early reaction." From this analogy it may be predicted that a repetition of these experiments in man will supply this last link, particularly since Bloch and Massini observed in man a typical "accelerated reaction" in one case. The earlier experiments with trichophytin established an analogy with the reaction observed in tuberculosis, and the latter experiments of Bloch and Massini established a close analogy with the train of events seen in vaccinia, and these authors recognized that the trichophyton infections give rise to an altered reactivity of the organism, the allergy of v. Pirquet. Following this idea, Bloch used the trichophytin for the cutaneous test and with success. Just as v. Pirquet showed that the cutaneous tuberculin reaction was positive in individuals suffering with tuberculosis or having passed through a tuberculous infection, patients suffering with a trichophytia of not too superficial a character gave a positive cutaneous test with trichophytin which could be elicited even long after the infection had disappeared.

Thanks to the kindness of Dr. Bloch in supplying us with his trichophytin, we were able to make a number of observations which will now be described.

*Trichophytins.*—Plato prepared his trichophytin by growing the fungi in a 3 per cent. maltose bouillon for two and three months at room temperature. The luxuriant growth is ground up as much as possible with a glass rod or strong platinum wire and filtered through a sterile filter-paper. Transfers from the filtrate to agar-agar proved the absence of living fungi. After an addition of 0.25 per cent. carbolic acid, the trichophytin is ready for use. Truffi prepared his trichophytins in a similar manner, but in order to insure their absolute sterility, he filtered through Chamberland candles and boiled the filtrates, or even heated them to 120° in the autoclave without destroying their activity. Furthermore, he was able to extract the active principle from a culture grown on solid media with absolute alcohol. The residue of the evaporated alcoholic extract was dissolved in water and proved active. Ether did not destroy its activity. Pautrier and Lutembacher took a culture grown for fifteen days in solid media, ground it up with serum and heated it at 110°.

Bloch and Massini filtered cultures grown in maltose bouillon through Chamberland candles and preserved the trichophytin by addition of 0.5–1 per cent. carbol-glycerin. The culture must be at least four weeks old. The activity is not diminished by keeping it two to three hours in the thermostat at 37°, and for a longer period of time in the refrigerator. Evaporation *in vacuo* at 40° does not interfere with its activity, but heating under pressure destroys it.

A primary toxicity of the trichophytins seems very doubtful. An experiment of Bloch may be mentioned with regard to the primary toxic effect of trichophytin. A bouillon culture of eight months standing was filtered through a Chamberland filter and the filtrate injected repeatedly, in quantities of 1–2 centimeters, intraperitoneally and subcutaneously in guinea pigs. Within three weeks the weight of the animals had dropped from 600 to 400 grams. He deduced from these experiments a toxic effect. But the condition of the experiments hardly justifies the assumption of a direct toxic action of the trichophytin, which is the substance responsible for the reactions. The vaccinations were made with the undiluted trichophytin, according to the method of v. Pirquet.

Bloch and Massini describe the reaction as follows: At the earliest after four hours, and usually after twelve to twenty-four hours, the reaction appears in the form of a flat red papule of the size of a lentil or pea, causing violent itching. In the following twenty-four hours the papule increases somewhat in size and recedes slowly in the course of the next days; but sometimes it remains for a longer time. Frequently a brownish pigmented spot is seen in its place for several weeks. This is the typical positive reaction. Sometimes and particularly in superficial cases, the papule is smaller and only a little elevated, that is, the reaction is weaker. Healthy individuals never give a positive reaction. The time at which the cutaneous trichophytin reaction occurs was determined by inoculating an individual (Dr. M.) with a small amount of a culture, and from the second day daily cutaneous vaccinations were made with trichophytin obtained from the same culture. The inoculation produced a typical skin lesion. The cutaneous reaction remained negative up to the sixth day. On the seventh day the vaccination elicited a small reaction. The vaccination of the eighth day produced a distinct papule. The vaccination of the following days led to a typical reaction which did not increase in intensity at the subsequent vaccinations. The reaction remains positive long after the disease has run its course, and in one case it occurred two and one-half years after the infection was cured. The intensity of the reaction was proportional to the intensity of the still existing or previously existing disease. The deeply infiltrating forms give the most intense reactions. The reaction was negative in cases where the infection limited itself to the hairs or the superficial horny layers of the epidermis. Cases with some infiltration give a moderate reaction.

Our own experiments were limited to cutaneous vaccinations with trichophytin. Unfortunately we did not have cases at our disposal where a trichophytosis still existed.

The total number of cases vaccinated with trichophytin was 131. The age varied from two weeks to seventy-nine years. The vaccinations were made according to the method of v. Pirquet, and two vaccination points were made with the trichophytin and one control.

The following table records the cases in which a history of

trichophyton infection was obtained and where the reaction was positive.

TABLE I.

*History of Trichophytosis and Positive Reaction.*

	Age.	History.	Remarks.
1. P. H.	61 years.	29 years ago ringworm on neck, lasting 9 months.	Seen every second day. On the sixth day reaction negative. On the eighth day definitely positive reaction. Papule of 5 mm. diameter, red, elevated. Two days later practically faded, hardly any redness, no elevation.
2. T. B.	67 years.	Ringworm on face and arms, several attacks; the last, 40 years ago.	On second day reaction faintly positive, little raised red papule. Two days later entirely faded.
3. T. D.	63 years.	13 years ago doubtful sycosis.	On second day one point, red, raised papule 6.5 mm. in diameter, the other point faintly, positive. On fourth day first point about same diameter, the other point red, raised papule of 5.5 mm. diameter, On sixth day about the same. Three days later nearly entirely disappeared.
4. Mr. T.	24 years.	When 6 years old, ringworm on face and chin; easily cured, no deeper lesions.	After 6 hours positive reaction, reaching its maximum after 24 hours with a diameter of 7 mm. The size, redness and elevation gradually decreasing within the next 6 days.
5. Dr. B.	29 years.	17 years ago ringworm on face, lasting more than one month. 7 years ago ringworm on genitals, reaching down the thigh half way to knee. Duration 3 to 4 months.	After 24 hours red, raised and slightly painful papule of about 5.5 mm. diameter.
6. N. H.	7 years.	Superficial ringworm on head; has been under treatment.	Microscopic examination did not show any mycelia in hair. After 24 hours red papule slightly elevated, and redness not very intense. Not seen any more.

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|------------|-----------|---|---|
| 7. S.      | 30 years. | When about 6 years old eruption on skin of face. Several other children had the same eruption. Lasted about one week. | After 21 hours red, raised papule increasing in size and intensity during the next 24 hours, gradually disappearing during the next week.<br>Second vaccination positive also.  |
| 8. Dr. As. | 36 years. | Dhobie itch in Philippines. Last attack 7 months ago. (No other infection with trichophytosis.)                       | First vaccination positive, very doubtful. Second vaccination 17 days later. After 24 hours red, raised papule about 5 mm. in diameter. 24 hours later diminished much in size and intensity, rapidly disappearing 24 hours later like control. |
| 9. A.      | 49 years. | Deaf mute.<br>No history obtainable.  | After 24 hours slight reaction, red and a little raised. 2 days later about the same. 3 days later nearly like control.   |

Of the 131 cases, eighty-two were inmates of the tuberculosis ward of the Bay View hospital,<sup>2</sup> and these were observed at two day intervals for at least one week. Seven cases were patients in the Johns Hopkins Hospital, Ward F, and could be observed daily for a sufficient length of time. Ten cases were taken from the children's ward of the Hebrew Hospital. Seventeen cases are represented by students and physicians working in the children's department of the Johns Hopkins University, and fifteen cases were patients in the same department. In these latter cases, the observation could not be continued as long as we might have wished, for frequently the patients did not return more than once or twice. A number of vaccinations could not be recorded at all because the patients either did not return, or returned after too great an interval. Of the 131 cases, 107 were vaccinated simultaneously with tuberculin, bouillon treated exactly like the tuberculin but without any bacteria, and trichophytin. The bouillon vaccinations were made in order to see whether it might give a reaction by itself. In one case only, a doubtful reaction occurred, and this was at the point where the bouillon was inoculated. All the other cases showed a completely negative result.

<sup>2</sup>I wish to acknowledge my indebtedness to Dr. L. F. Barker and Dr. J. Wilson for their permission granting me access to the wards of the Johns Hopkins Hospital and Bay View Hospital.

Twenty-four hours after the vaccination, the measurements were as follows: tuberculin, 4:4 mm., 5:4 mm.; bouillon, 4:4, 4.5:5 (redder and a little more raised than tuberculin and trichophytin); trichophytin, 4.5:3.5, 4:4; control, 3:2.5 mm. Twenty-four hours later: tuberculin, 3:4, 4:3; bouillon, 4:4.5, 5:4; trichophytin, 3:3, 4:4; control, 2.5:2.5. The bouillon vaccination points showed the most pronounced changes, being somewhat more red and elevated than the others, although the reaction was not a very definite one. Twenty-four hours later all the vaccination points were alike. This reaction is given in full, because it was the only one where any doubt was possible with regard to a positive reaction elicited by the bouillon alone.

Besides these 107 cases, ten were vaccinated with tuberculin and trichophytin, one with bouillon and trichophytin, and thirteen only with trichophytin.

It may be remarked that the trichophytin is much darker than the bouillon or the tuberculin, and frequently a pigmentation was seen around the points of vaccination, sometimes arranged in ring form, disappearing in a few days. This pigmentation differs from the pigmentation which is left on the place of vaccination after the disappearance of a positive reaction, and it is due no doubt to the pigment contained in the trichophytin. The tuberculin vaccinations did not give any results not noted before.

Of interest are the two cases giving a history of Dhobie itch (Table I, No. 8; Table III, No. 4). Both were physicians who had spent some time in the Philippines. The first vaccination was negative in both cases; in one a second vaccination could be given seventeen days after the first. Now the reaction, although slight, was definitely positive. The Dhobie itch is, according to Manson (10), a disease not of uniform etiology, but in a certain number of cases hyphomycetes belonging to the trichophyton group seem to play a rôle. The cutaneous test with trichophytin may be of value in distinguishing this group from others due to different infections.

In any case it would be desirable to extend the investigations in this direction. In the one case giving a positive reaction on the second vaccination, it is very probable that here we have an induced reaction like those seen in tubercular individuals where the amount of antibody is insufficient, but where the vaccination stimulates the production of antibodies, so that after a first negative result, the subsequent vaccinations are positive. One other negative result was of special interest. A colored child, eleven months of age, came to

the dispensary with an affection of the skin on the forehead and around the eyes. The center of this affection had cleared, while it was spreading with an infiltrated, slightly inflamed margin. The disease had lasted for some time, withstanding treatment by different ointments. The trichophytin reaction was negative and microscopically no hyphomycetes could be detected in some material taken from the margin. The lesions disappeared entirely under antisyphilitic treatment. There were no other signs of syphilis to be found. Another case deserves special mention. A man 61 years of age gave a history of having had a ringworm 29 years ago. The vaccination points with trichophytin did not show any sign of reaction on the sixth day; on the eighth day there was a definite although slight reaction (Table I, No. 1) which had disappeared two days later. This reaction is analogous with the accelerated reaction seen in cases of vaccination in vaccinia.<sup>3</sup> Similar late reactions like this are seen, although rarely, in tuberculin vaccinations. The explanation in both cases is that the organism has an insufficient amount of antibody at its disposal to react immediately with the vaccine or the tuberculin. The vaccination leads to a renewed formation of antibody which reacts with the vaccine or tuberculin or trichophytin left on the place of vaccination, producing the toxic substance giving rise to the reaction. In this way the slight, rapidly disappearing reaction of our case could be explained, since it is very unlikely that after the lapse of several days much unresorbed trichophytin should have remained on the place of vaccination. In the case of tuberculin such late reactions are extremely rare, and it requires a second vaccination to show this secondary reaction, because none of the tuberculin seems to be left at the place of vaccination. For the same reason, it is not probable that such a late reaction will be seen frequently in the case of trichophytin. In vaccinia the accelerated reaction on relatively late revaccination (corresponding to this type of reaction just described in trichophytin and tuberculin) is a rather regular occurrence and for a very simple reason. Here the allergen consists of living

<sup>3</sup> See v. Pirquet, Fig. 23 in "Klinische Studien ueber Vakzination, etc.," Leipzig, 1907. In this figure there occurs a misprint. The lines recording the events in first vaccinations and revaccinations are interchanged.

organisms which even multiply. Whenever now the renewed formation of antibody has reached a sufficient concentration, it will always find sufficient allergen at the point of vaccination to produce the toxin.

Bloch states that as far as his experience goes, the cutaneous reactions were never positive in cases which did not have a trichophyton infection. We have four cases (Table II) where the reaction was positive, while there was no history obtainable of any previous skin disease. Two of these cases (Nos. 1 and 2) were 39 and 54 years old, both inmates of the Bay View Hospital, and the correctness of their data may be open to doubt. But the two other cases (Nos. 3 and 4) were students, and one of these took special pains to find out whether in his early life a skin affection had been noticed. As far as could be ascertained, in both these cases there had been no skin affection. At present we will refrain from drawing any conclusions from these observations, being satisfied with the statement of the facts as they presented themselves. Of the four cases giving a history of ringworm with negative reaction (Table III), one was a case of Dhobie itch, and this disease is not necessarily due to a trichophyton infection. One case was a man 72 years of age, giving a history of ringworm when young; the other was a

TABLE II.

*No History of Trichophytosis and Positive Reaction.*

	Age.	History.	Remarks.
1. W. B.	39 years.	Does not know of any scalp, beard or skin disease.	After 48 hours exudation, hardly any inflammation. 48 hours later, red, raised papule about 8 mm. in diameter gradually disappearing within the next week.
2. S. S.	54 years.	Same history.	After 48 hours red, raised papule about 8 mm. in diameter disappearing within a week.
3. Mr. Tr.	25 years.	No known infection with trichophyton. Parents state that he had never any skin affection.	After 24 hours red, raised papule 5 mm. in diameter. During the next days increased in size and intensity, itching, gradually disappearing with scaling. Second vaccination, 69 days later, positive.

4. M. R. 26 years. No known trichophytosis. After 24 hours little red and raised papule of about 6 mm. in diameter. 24 hours later about same size but somewhat more intense.

TABLE III.

*History of Trichophytosis and Negative Reaction.*

	Age.	History.	Remarks.
1. C.	55 years.	20 years ago skin eruption on leg, of short duration. Doctor called it ringworm.	No reaction within 9 days.
2. B.	64 years.	Occasional attacks of ringworm when quite small. Cured at home with green walnuts.	No reaction within 2 weeks.
3. J.	72 years.	Ringworm when young.	No reaction within 2 weeks.
4. M. P.	32 years.	Dhobie itch in Philipines 4 years ago. Very intense. (Never any other trichophytosis.)	No reaction.

man 64 years of age, giving a rather doubtful history of ringworm when quite small; and finally, there is the case of a man 55 years of age, having had a skin eruption on the leg for twenty years, previously diagnosed as ringworm. In these cases the time elapsed between infection and vaccination may have been sufficient to permit the disappearance of the antibodies, even if there should have been a trichophytia profunda, or supposing the skin disease really due to a trichophyton infection, it may have been a more or less superficial one.

With regard to the cases giving a history of a skin affection possibly due to a trichophyton infection with a positive reaction, a few are well substantiated. A detailed discussion of the others would lead us too far. Suffice it to say that more detailed data are desirable.

Dr. B. (Table I, No. 5), giving a definite history of trichophytosis, showed a positive reaction. He was first vaccinated on December 18, 1909, and for the second time on December 20. Twenty-four hours after the second vaccination a faint, indistinct,

red halo was seen, very irregular in shape, surrounding the sharply defined red and raised papule. This halo had disappeared about thirty-six hours after the vaccination. On March 2 the vaccinations were again commenced with the intention of increasing the reactivity as much as possible by repeated vaccinations. Until March 11 Dr. B. was vaccinated four times, and each time the halo formation was pronounced. The vaccinations were made on the right and left arm, twice separately and twice simultaneously. The first vaccination on the left arm was definitely less intense than that on the right, which had been vaccinated before, while the subsequent vaccinations were alike in intensity. On March 14, at 9.30 A. M., Dr. B. was vaccinated on the right thigh with different dilutions of trichophytin; with each dilution four points were made in exactly the same manner described by v. Pirquet in his experiments with tuberculin. From the knee upwards the first point was a control without any trichophytin, the next row was made with a dilution of 1:256, the next with 1:64, the next with 1:4, the next with 1:2, and the last with undiluted trichophytin. In certain intervals the reactions were measured and their condition noted. From the measurements of the reactions with the different dilutions, an average was calculated and the values thus determined were used for the construction of the curves. The reactions were measured by marking their extent in two directions with pen and ink, and measuring the distance between the points thus marked.

The following protocol illustrates the manner of recording. It was taken twenty and one-half hours after vaccination and represents the highest development of the papules noted in our measurements. The order in which the reactions are given is from the inside of the thigh to the outside.

Control 2:2 millimeters.				
1/256	4.5:5	3.5:3.5	5:4.5	4:3.5
	Fading; redness diminished; induration diminished.			
1/64	5:5	4.5:4.5	6.5:5	7.5:5
	Tender, raised, red and indurated.			
1/16	7:7.5	7:7	6:6.5	8:8
	Tender, red, raised and indurated.			
1/4	9.5:8.5	8:8	10.5:9.5	8:8.5
	Very tender, much raised and indurated; intensely red.			
1/2	9:8	11:10.5	10:8.5	10:9

Very tender, much raised and indurated; intensely red.  
 I 12: 10.5 9.5: 11.5 11: 11 10.5: 10  
 Very tender, much raised and indurated; intensely red.  
 All reactions rather well circumscribed; no halo formation.

The following table represents the average measurements of the reactions at different times.

Vaccination 9.30 a. m.	March 14.				March 15.			March 16.		Mar. 17.
	1.30 p. m.	5.30.	9.30.	12 night.	6 a. m.	12 noon.	7.30 p. m.	12 noon	11 p. m.	12 noon.
Hours after vaccination	4	8	12	14½	20½	26½	34	50½	61½	74½
Dilutions.	Average Measurements in mm.									
I : 256	2	3	4.2	4.2	4.2	3.5	3.5	2.4	2	2
I : 64	2	2.5	4.4	4.6	5.6	5.6	5.3	2.6	2.8	2
I : 16	2.2	4	6	6.2	7.1	7.3	13.2*	6	5.3	3.6
I : 4	2.5	4.1	5.8	7.1	8.8	9.2	14.6*	11.7†	5.6	4.3
I : 2	2.8	4.7	6.9	7.8	9.5	9.1	19.6*	6.2 16.3 6.9	7.6	4.6
I	2.1	5.1	8.3	9.8	10.8	9.4	23.9*	20.6 6.4	8.4	5.4

The figures marked \* in the table represent the measurements of the halo surrounding the papule. The one figure marked † is the average of two areas, those of the other two points of this series having disappeared. In the series 1:2 and 1 it will be seen that the measurements of the papule showed again a slight increase after 61½ hours. At this time the elevation, induration and intensity of the redness of the papule had diminished to such an extent that it was very difficult to distinguish the papule from the ill-defined remainder of the halo. These measurements, therefore, include the rest of the halo and were not taken into consideration in constructing the curves of the papules. Between the last measurements of March 15 and the first of March 16, the rather long interval of 16½ hours had elapsed. This omission was due to the fact that with the development of the halo Dr. B. was taken sick.

On March 15, about twenty-four hours after the vaccination, the first signs of a halo around the papule appeared in the series 1:2 and 1. During the afternoon this halo became more and more pronounced until in the evening, thirty-four hours after vaccination, the halo from 1:16 to 1 had assumed a bright red color, not appreciably differing from the papule, so that the papules could not be measured separately. In 1:2 and 1 the halos of the neighboring points were running together at different points, so that the meas-

urements of the halo in one direction are not quite exact. At the same time Dr. B. complained of severe headache, fever, general muscular pains, and particularly pain in the vaccinated leg, com-

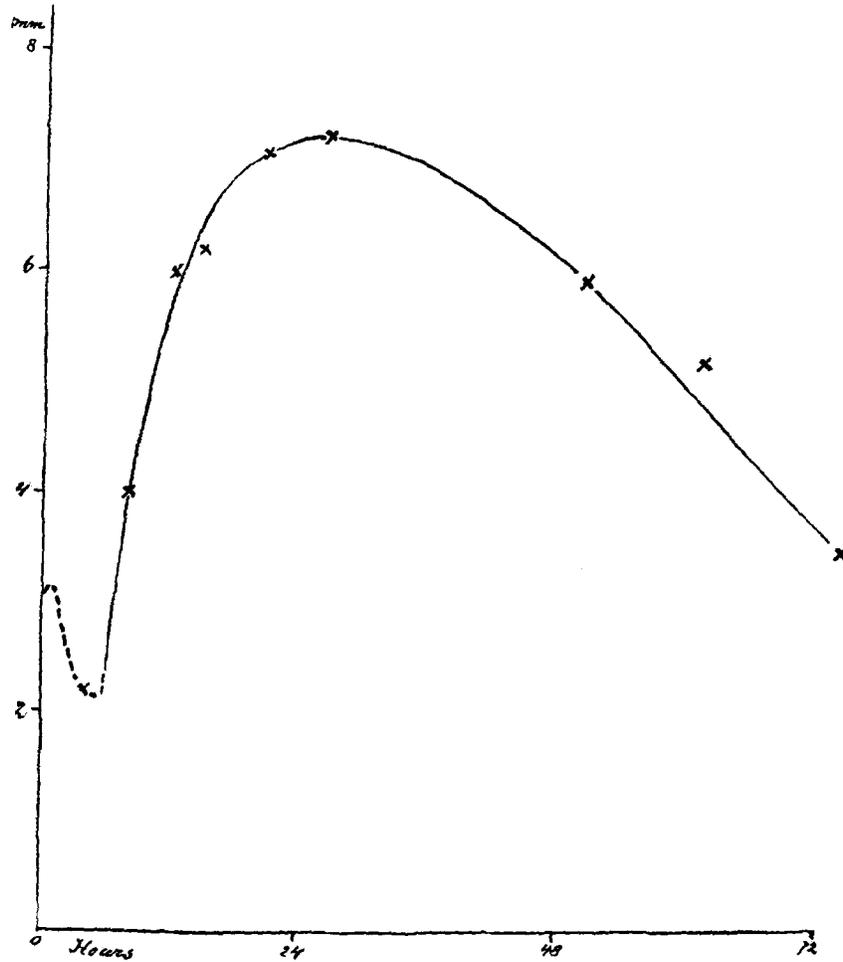


FIG. 1.—Dilution of trichophytin, 1:16.

plete loss of appetite, violent throbbing in the head, slow heart action and painful swelling of the right inguinal glands. During the night itching on the field of vaccination added itself to the symptoms. Dr. B. felt too poorly to make any observation during

the night. The next morning he had improved somewhat and noted that there was apparently no change in the size of the reactions, but that the intensity had diminished very considerably. At noon on March 16 the general pains had disappeared almost entirely, as well as the headache, the reactions were less painful and the inguinal glands had diminished but were still tender on pressure. The halos of 1:2 and 1 were rather well defined, though much less red than the papules. In the series 1:4 only two halos were still to be made out and one other was very indefinite. In the series 1:16 only one of the reactions presented a very indefinite halo. In the course of the day Dr. B. recovered completely and the remaining lassitude had left the next day.

The course of the reaction may be best illustrated by the curves, which were constructed according to the method of v. Pirquet. A system of base line and ordinates was marked on quadrille paper, where the base line represents the hours and the ordinates the size of the reactions, and since the size of the reactions corresponds directly with their intensity, the ordinates represent the intensity of the reactions. This system served for the preparation of all the curves, the paper on which the curves were drawn was laid on top of it, and the figures were marked in their proper places. Then the curves were drawn in such a manner that the line passed through as many points as possible. Fig. 1 illustrates the manner in which the curves were drawn. The curve represents the development of the papule with a dilution of 1:16. The control was not measured until twelve hours after the vaccination, when it had reached the level on which it remained; therefore, it was not possible to mark the traumatic reaction which always follows any vaccinations. The beginning of the reaction proper, therefore, cannot be marked with accuracy in the curves. From the close analogy of our curves with those obtained by v. Pirquet in his experiments with tuberculin, it was thought permissible to indicate the traumatic reaction in our curves as though it were analogous to his findings. To indicate that this part of the curves is not based on our figures, it is drawn in broken lines. Comparing our results with those obtained by v. Pirquet in his experiments with tuberculin, we see by a glance at Fig. 2, representing all the curves, that as in the case of

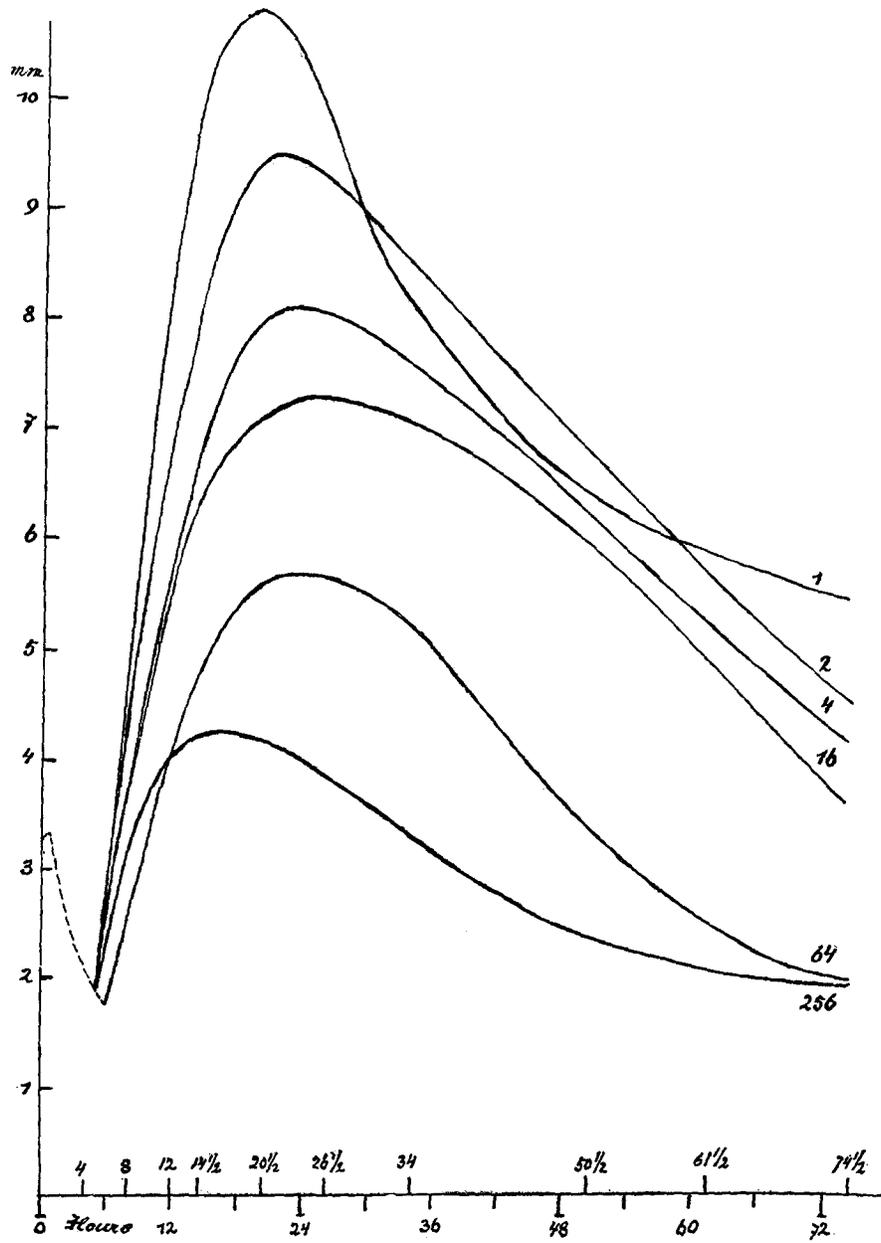


FIG. 2.

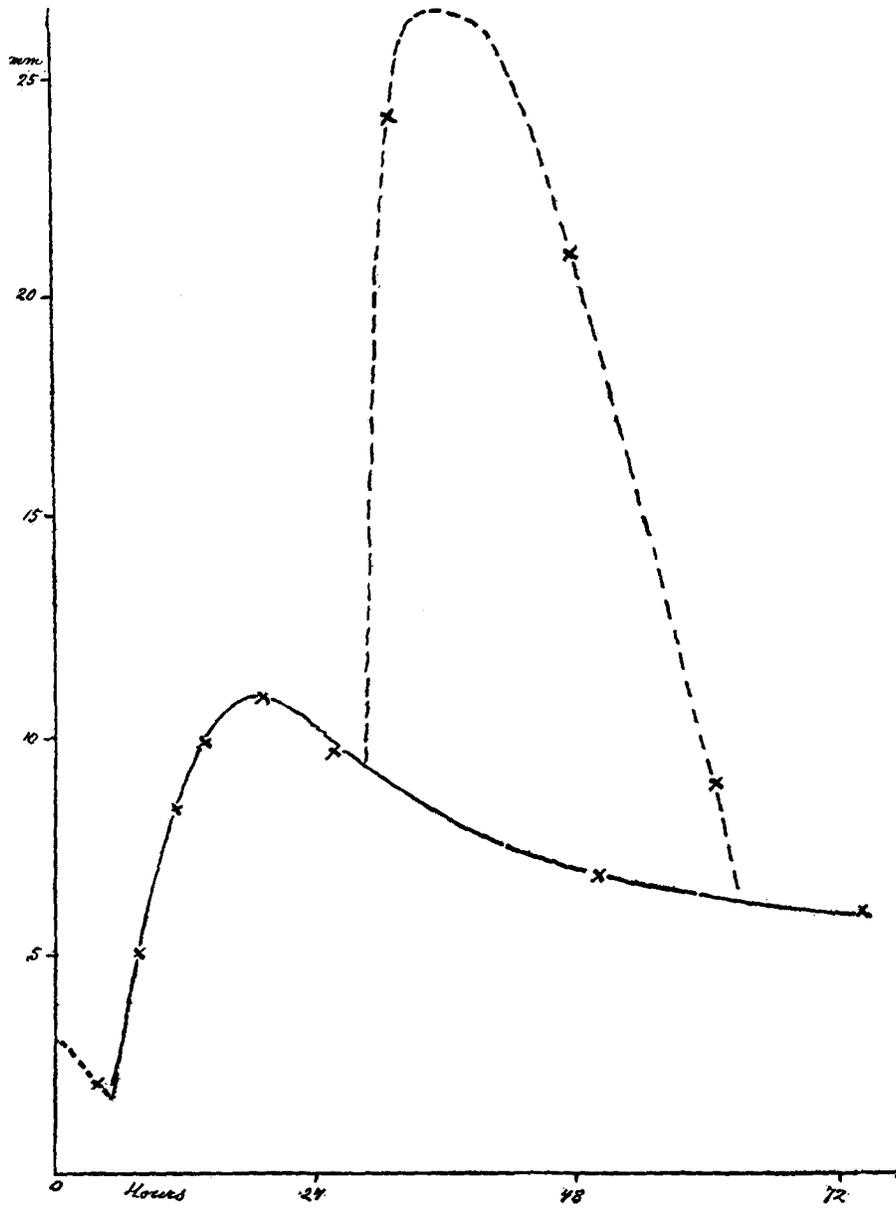


FIG. 3.—Dilution of trichophytin, 1:1.

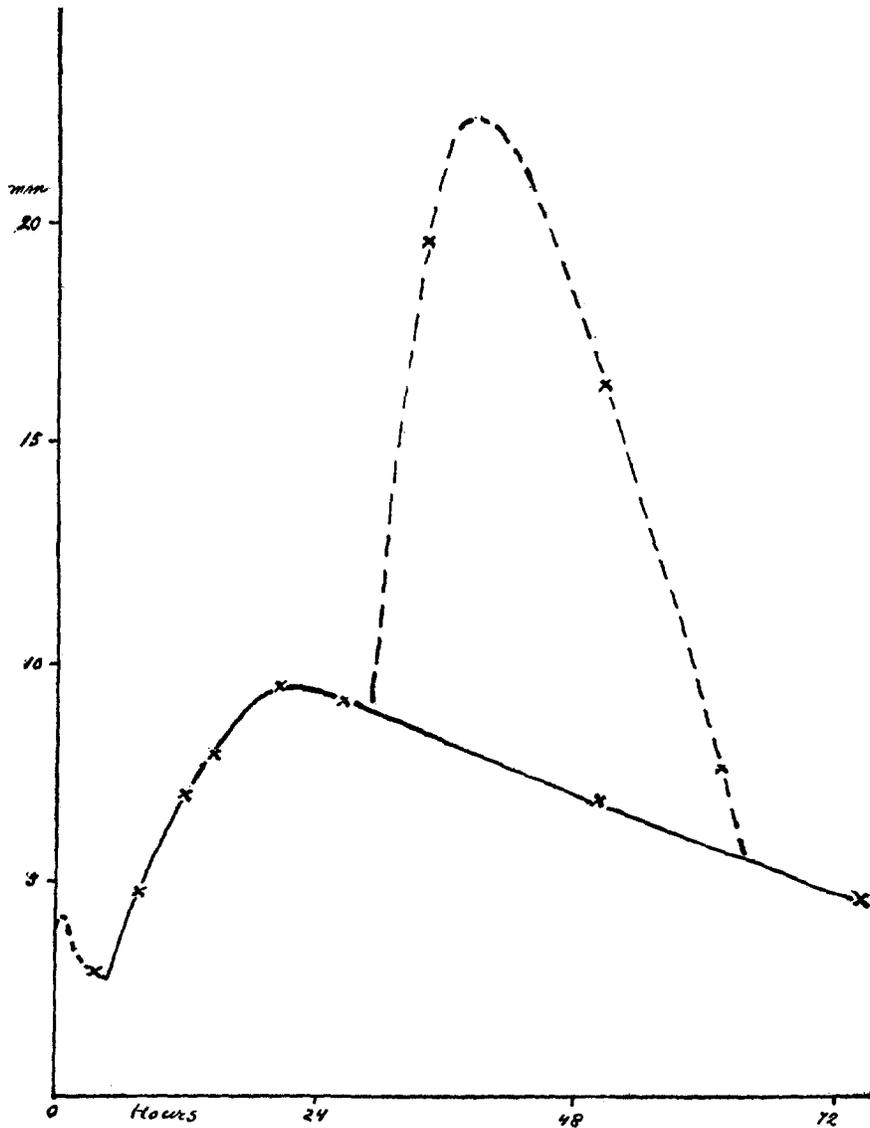


FIG. 4.—Dilution of trichophytin, 1:2.

tuberculin, the intensity of the reaction is directly proportional to the concentration of the trichophytin.

The curves in Figs. 3, 4 and 5 are of special interest. The lower

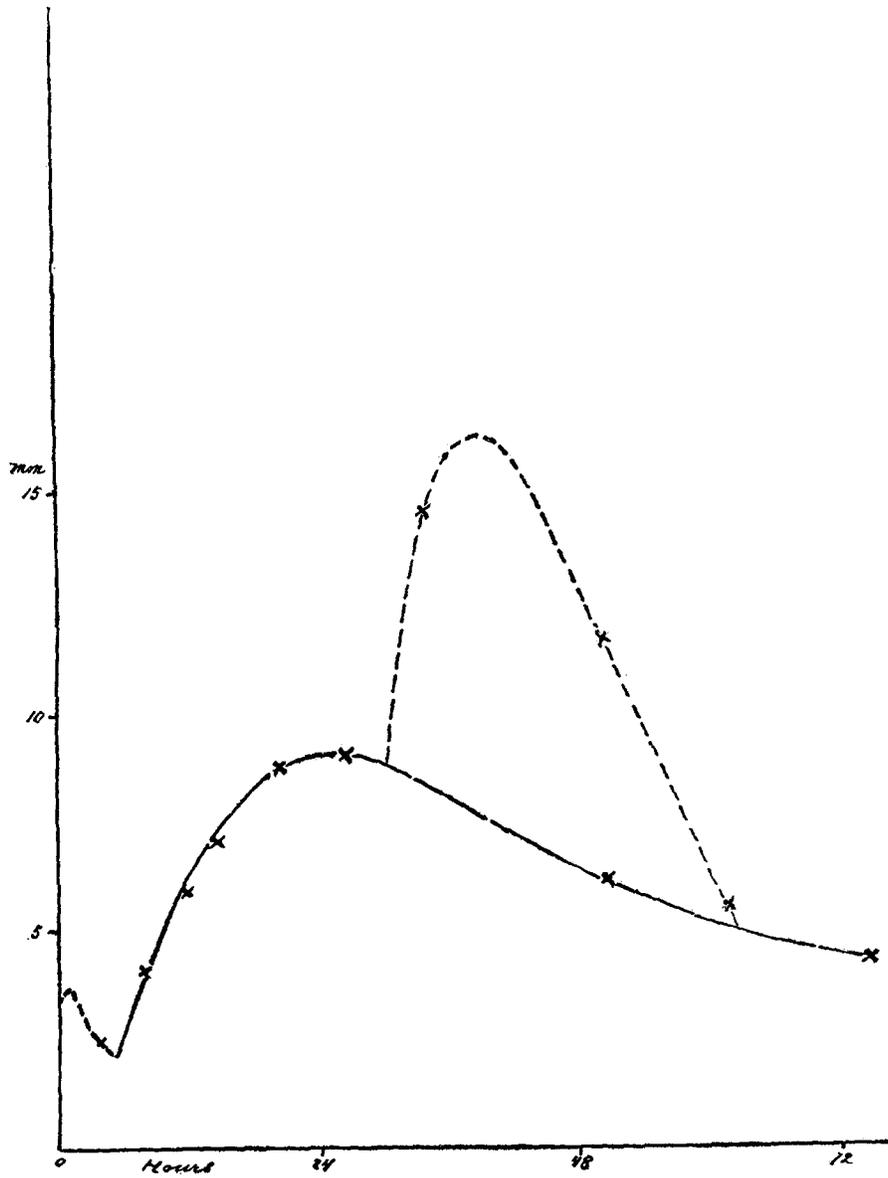


FIG. 5.—Dilution of trichophytin, 1:4.

curve drawn out represents the size of the papule, while the superimposed curve drawn in broken line represents the size of the halo.

The first signs of a halo were seen in some reactions about twenty-four hours after vaccination. Twenty-six hours after vaccination one point in the series 1:2 and all of the series 1 showed slight and very ill-defined indications of a halo; after thirty-four hours all the points of the series 1:16 were surrounded by an intense halo; after fifty and one-half hours the halos of the series 1:16 had disappeared entirely; in the series 1:4 only two were still visible and all had practically disappeared after about sixty-one and one-half hours. The starting point of the curve representing the halos, particularly in the series 1 and 1:2, should have been a little more advanced. Since the interval between the measurements was rather long, the acme of the curve was not fixed at the time of the measurement, thirty-four hours after vaccination. The time of the onset of the halo again shows a striking analogy with the results obtained by v. Pirquet in the tuberculin vaccination.

Thirty hours and thirty minutes after vaccination he notes: "Now it (the papule) is surrounded by a slightly red halo. The papule itself is smaller than in the previous measurements." This result coincides with those we have obtained as a glance at the curves will show. Here too the beginning of the halo is contemporaneous with the beginning involvement of the papule. Thirty-two hours and twenty-five minutes after vaccination the halo formation in v. Pirquet's experiment was very distinct. After 38 hours the halos were still larger. After 45 hours and 35 minutes the halo could only be seen indistinctly in a few places, but after 53 hours some places still showed a halo presenting in great part a very large (up to 35 millimeters) indefinite reddening. Three days after the vaccination the halos had disappeared entirely. The agreement of v. Pirquet's results with our results is certainly most striking.

Plate XXXIX, Fig. 1, illustrates the appearance of the reactions in our case. It was taken about fifty hours after the vaccination, when the papules were already in the process of involution, rather easily separable from the halos, and the halos were relatively sharply defined, although less sharply than represented in the drawing.

In v. Pirquet's experiment the development of the halos was not associated with a general reaction, at least none was noted. In our case the general reaction was so intimately associated with the development of the halos that we are justified in assuming a connection between these two reactions. A general reaction after tuberculin vaccination has been observed very rarely and no particular attention has been paid in these cases to the halo formation.

The analogy of the halo formation in our trichophytin vaccination with that in tuberculin vaccination consists in the time relation.

For this phenomenon the following explanation may be offered. The introduced trichophytin meets with a certain amount of antibody and the result is the immediate reaction with the formation of the papule. The intensity of the reaction depends, as we have seen, on the concentration of the trichophytin. The intensity of the halo formation also depends on the concentration of the trichophytin, for instance 1:256 and 1:64 do not form any halo at all, and with increasing concentration of trichophytin, the intensity of the halo increases, as may be seen from the table registering the average measurements of the reactions and Figs. 3, 4 and 5. It is conceivable that the concentration of the antibodies is sufficient to combine with all the trichophytin absorbed from the higher dilutions, while it is not sufficient to combine with all the trichophytin of the more concentrated solutions. The trichophytin—the allergen—left over spreads itself in the lymph spaces, a renewed production of antibodies takes place, and with the advent of these, the reaction is again renewed with the formation of the halo. The objection may be raised that under such conditions the first reaction should reach the same degree of intensity from a given dilution on. But the possibility exists that according to the law of mass action with increasing concentration of the trichophytin and uniform concentration of the antibody, a larger amount of the toxic product is formed. If the reaction of antibody and trichophytin follows the law of mass action, and we suppose a relatively large excess of trichophytin, this increased formation of toxic substance would not exclude the possibility that in spite of it with increasing concentration of the solutions a greater excess of trichophytin is left over. This explanation would cover the halo formation in both the tuberculin and the trichophytin reaction.

The intense general reaction of Dr. B. contemporaneous with the halo formation has still to be explained. The rapid development of the halo and its intensity indicate a rapid production of toxin. The larger area over which this reaction was spread may offer a better chance for a quick resorption of the toxic substance, which would then enter the general circulation in greater concen-

tration. The fact that in the tuberculin experiment no general reaction was noted may be due to a lesser toxicity of the toxic substance formed or to a greater individual sensitiveness of Dr. B.

In cowpox vaccination the general symptoms coincide with the formation of the area, while at the same time the involution of the papilla begins. v. Pirquet (12) brought forward strong evidence that the formation of the area and the general symptoms are due to the interaction of the produced antibodies and vaccine lymph with resulting toxic substances. There exists a seeming resemblance between a first cowpox vaccination and the reaction observed in our case. The area formation closely associated with the onset of general symptoms would be analogous to the halo formation, while the papilla beginning its involution at this period would seemingly correspond to the papule. But the phenomena observed at revaccination make it self-evident that this analogy is not a real one. Our type of reaction belongs to the "early reaction." The early reaction in cowpox vaccination leads to the formation of a papule, while the area formation does not take place any more. This papule is analogous to the papule of the tuberculin and trichophytin reaction and it really corresponds to the area of the first vaccinated.

It may be mentioned that the early reaction of cowpox vaccination shows another analogy to the tuberculin and trichophytin reaction, for v. Pirquet (13) showed that its intensity depends on the amount of introduced virus, like that of the tuberculin and trichophytin which is dependent on the concentration of the solutions. In the case of a first vaccination the intensity of the reaction is not dependent on the amount of virus originally introduced.

The formation of the area, as well as that of the papules, depends on the presence of antibodies, but in the first case the production of the antibodies requires some time during which the microorganisms of the lymph multiply and spread themselves to some extent in the surrounding tissue. Before the advent of the antibodies, the allergen increases constantly, and when the antibodies arrive, the concentration of the allergen is sufficient to elicit a violent reaction producing an area with general symptoms. In the early reaction, the organism, due to a previous infection, is already

in possession of a sufficient amount of antibody to react immediately with the introduced allergen, just as in the tuberculin and trichophytin reaction, only perhaps with the difference that at first substances analogous to the tuberculin or trichophytin must be liberated. There is no time left for the multiplication of the microorganisms, that is, for an increase of the allergen. The result is a relatively insignificant reaction with only a papule formation. The formation of the papilla is dependent and proportional to the multiplication of the microorganisms of the lymph. Therefore, it is obvious that there is an essential difference between the papilla and the papule of the early reaction. That nothing corresponding to our halo formation is seen in the early reaction of cowpox vaccination can be easily explained. An early reaction in this case means a sufficient concentration of antibodies to inhibit the growth of the specific microorganisms immediately. The concentration of the antibody is relatively great when compared to that of the allergen, which did not have time to increase, while for the formation of the halo it seems necessary that the active mass of the allergen exceeds that of the antibody.

From our experiment the conditions for the formation of a halo are the following: Sufficient concentration of the antibody to produce an early reaction and excess of introduced allergen. The allergen left over spreads in the lymph spaces, stimulates a renewed production of antibodies, and this renewed production of antibodies proceeds rapidly, resulting in a rapid rise of the reaction. With a change of concentration of the antibodies on one side and that of the allergen on the other, we may naturally expect different degrees of halo formation and changes in its time relation. That some of the trichophytin may remain at the place of vaccination for a long time is seen in the case described, where the reaction did not show itself before the sixth day. According to the conceptions of v. Pirquet, it is very probable that the whole organism participates in the cutaneous tuberculin reaction, although in the great majority of cases the manifestations remain below the threshold of clinical observation. The renewed production of toxic substance in our case may have sufficed to bring these manifestations above the level of clinical observation in a specially sensitive individual.

Our explanation of the halo formation does not seem to agree well with the observation of v. Pirquet in his experiment with tuberculin vaccination. Here all the vaccinations varying from a dilution of 1:512 to undiluted tuberculin showed a halo formation, and even the control participated in the hyperemia. No data are given about the intensity of the halo formation with the different dilutions. The participation of the control itself cannot be explained at present. That the higher dilutions participated in the halo formation may be due to the fact that the tuberculin contains the allergen in greater concentration than the trichophytin.

Unfortunately we did not have the opportunity of testing acute cases of trichophytosis, but from the description of the cutaneous reaction given by Bloch and Massini it seems that the reaction was never as intense as is frequently seen in the tuberculin reaction, as we had the opportunity to see in the inmates of the tuberculosis ward at Bay View. We, therefore, may assume that the concentration of the allergen in the tuberculin is really greater than that of the allergen in the trichophytin. The method of preparation of tuberculin and Bloch's trichophytin would speak also in favor of such a view.

Our explanation of the halo formation is tentative, and we are well aware that it needs further investigations to establish it on a firmer basis.

#### CONCLUSIONS.

There exists a far-reaching analogy between the cutaneous trichophytin reaction and the cutaneous tuberculin reaction. Both indicate that the organism is the seat of a definite infection or that it has passed through such an infection.

Both may persist for a long time after the active disease has come to rest, indicating that the infection has left the organism in a state of altered reactivity—allergy.

Under certain conditions both may be of diagnostic value, but since the reaction persists for a long time after the infection has passed, the negative reaction may be of greater value, excluding the existence of a specific infection.

The analogy of the trichophytin reaction with the tuberculin reaction is not only limited to the obvious clinical manifestations, but, as in the tuberculin reaction, it can be shown that with uniform concentration of antibody, the intensity of the reaction is dependent on the concentration of the trichophytin.

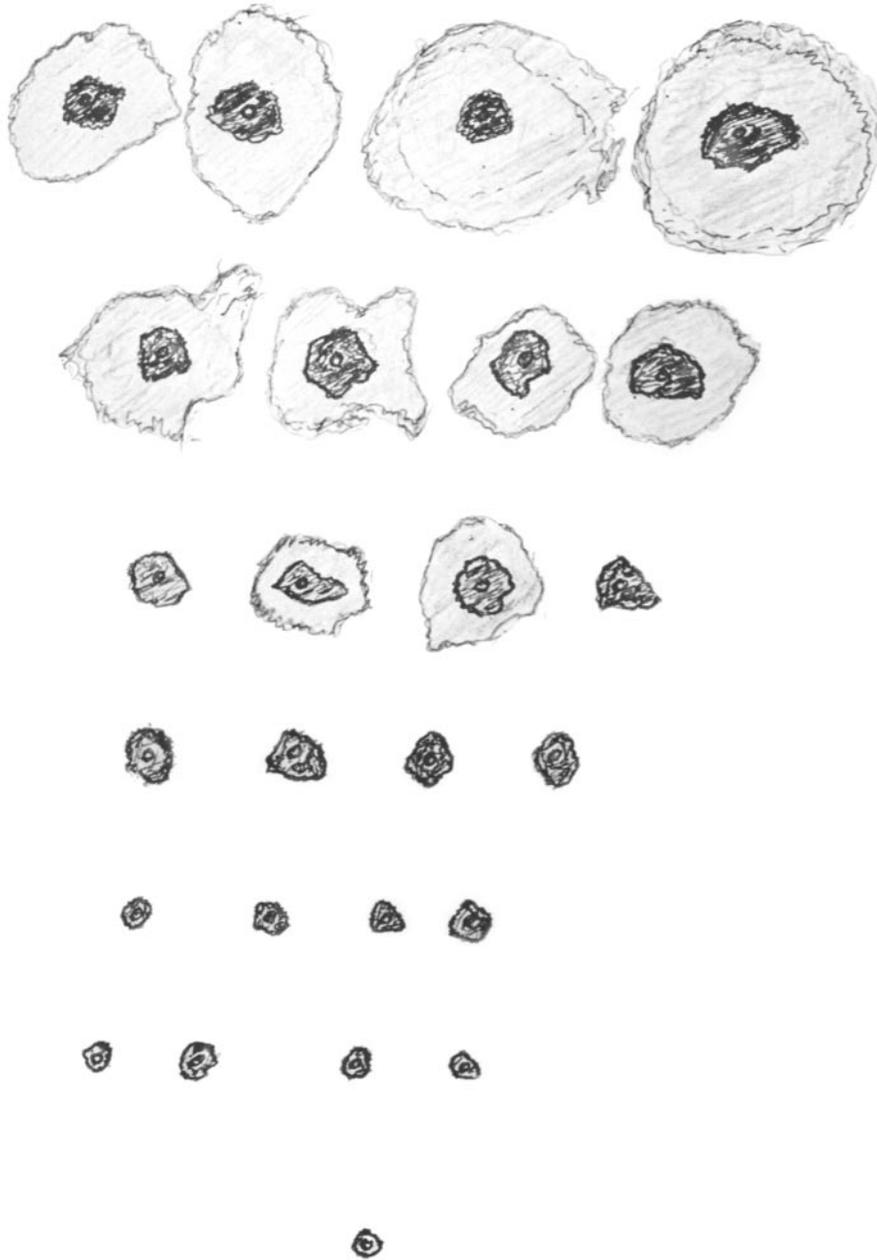


FIG. 1.

A tentative explanation of the halo formation is offered, based on a rapidly renewed formation of antibody stimulated probably by the entrance of a small amount of allergen into the general circulation.

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