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FORMOLIZED HERPES VIRUS THERAPY AND THE NEUTRALIZING SUBSTANCE IN HERPES SIMPLEX[1]

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Since the demonstration of the viral etiology of herpes simplex, there has, naturally, been considerable interest in the immune responses to this virus and particularly the ability of serum obtained from humans and animals, exposed to herpes, to neutralize a known virus.

The purpose of this study was to treat patients suffering from recurrent herpes simplex with a formalin inactivated herpes virus and to study quantitatively the antibody response (i.e. the neutralizing titre of the blood-serum) to these injections and, also, to natural infection.

It is a well established fact that rabbits and guinea pigs that have recovered from experimental herpetic infection show a degree of immunity roughly parallel to the neutralizing property of the serum, i.e. hyperimmunized animals, showing higher titres in their sera, remain immune longer and resist higher concentrations of virulent virus.

This is in marked contrast to man, where the unusual circumstance is found of a viral infection recurring with undiminished virulence in spite of neutralizing antibodies in the sera. Eruptions frequently—in fact, usually—recur at or near the same site,

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indicating that no local immunity is produced. Quantitative studies of the antibody titre in relation to recurring attacks have not been made. Many investigators have studied the incidence of neutralizing antibodies in the sera of "normal" persons and those with herpes. Gay and Holden (1) found specific neutralizing antibodies in the sera of four out of nine individuals with occasional attacks of herpes, and five out of seven with recurrent herpes. Andrewes and Carmichael (2), (1930) observed these antibodies in the sera of each of seven cases of recurrent herpes and in 75 per cent of controls. Zinsser and Tang (3) found that 58.8 per cent of sera from normal adults and 43 per cent from children contained this protective substance. Weyer (4) has shown a correlation between age and the development of the antibody: 0—5 years, 14 per cent; 20–25, 90 per cent; over 45, 54 per cent. Hudson, Cook and Adair (5) were unable to show any appreciable alteration in incidence of neutralizing antibodies in relation to sex, pregnancy or menses.

In reference to the treatment of recurrent herpes simplex, Gildermeister and Herzberg in 1925 (6) and 1927 (7) reported that there was a partial cross immunity between the viruses of herpes and vaccinia. However, this observation was counter to the experience of the earlier workers with these two viruses. Bedson and Bland (8), and others, were unable to confirm the observations of Gildermeister and Herzberg, and it is generally conceded today, that there is no such relationship between these two viruses. The observations of Rivers (9), that skin which has just reacted tends to be less reactive, must be kept in mind; for example, measles prevents the appearance of a Dick test. This might be an explanation for Gildermeister and Herzberg's findings. However, in 1928, Freund (10) treated seven cases of recurrent herpes with two vaccinia inoculations and reported clinical freedom from recurrences. Minami and Ohmichi (11), using a single inoculation of vaccinia in 10 patients reported satisfactory results in all of them. In 1934 Wise and Sulzberger (12a) reported satisfactory results with repeated vaccinations in some but by no means in all their cases of recurrent herpes treated by this method. Recently, Foster and Abshier (12b),

using 2 to 6 vaccinia inoculations in patients with recurrent herpes, reported their results on 35 patients followed over a period of two years. Of these 35 patients only 5 had recurrences. R. T. Brain (13) in discussing the apparent clinical results obtainable in treating recurrent herpes simplex with small pox vaccination expresses the concept that this effect is "non-specific." Foster and Abshier take exception to this concept and feel that there is a group immunity phenomenon involved.

FORMOLIZED HERPES VIRUS USED THERAPEUTICALLY

Several workers have attempted to approach the problem of treating these troublesome cases of recurrent herpes with a more direct method. The herpes virus can be communicated to both the rabbit and guinea pig, as well as to other animals, with facility. Hence various clinical attempts have been made to use preparations derived from animal tissues infected with the virus, treated with phenol, formalin, or heat, in such a way that the virus can be termed "killed," i.e. the treated material could no longer infect a susceptible animal. Holden (14), working with rabbits, and Bedson (15), with guinea pigs, have been able to produce immunity by means of a formalin-treated virus.

Martin and Caneja in 1933 (16) used a phenol-killed brain emulsion intradermally in five cases of recurrent herpes of the cornea. Two of these cases appeared to respond to this therapy and experienced cessation of recurrences after the eighth and seventeenth injection, respectively.

Biberstein and Jessner, in 1935 (17), used a 10 per cent heat-killed emulsion of infected rabbit brain. Two-tenths of a cubic centimeter of this material was injected intradermally twice weekly for several weeks. Of eleven patients treated with recurrent herpes simplex nine showed some therapeutic effect, i.e., a definite lengthening of interval between attacks and a decrease in severity of these attacks. These authors also observed in some of their cases a displacement of the lesions to a new site, a focal reaction following the first injection, and herpetic-like reactions at the site of the intradermal inoculations (Isomorpher Reizeffect of Koebner?).

R. T. Brain (13) in 1936 reported five cases of recurrent herpes treated with a ten per cent suspension of infected guinea pig pads inactivated with formalin. Each patient received weekly subcutaneous injections of 0.5 cc. to 1 cc. over a period of one to seven weeks (maximum given to any one patient was 7.1 cc.). Brain also found an increase in interval and decrease in severity of the attacks in the treated patients.

Merrill (18) has pointed out that there is a definite relationship between the size and number of antigenic particles necessary to produce demonstrable immunological reactions, and has suggested that this mass-factor be considered in immunologic studies on virus.

It was, therefore, desirable to follow the effects of large doses of a formolized herpes virus given over a long period of time. This work was started in February 1936. During this period 14 cases were followed. Every care was taken in the selection of patients used in this series. Only patients who had attacks at regular rhythmic intervals were selected, i.e. at least one attack in eight weeks. Every effort was made to ascertain the frequency of attacks before treatment was instituted, inasmuch as, ordinarily, patients are apt to be unreliable as to the frequency of attacks. In the cases where the lesions or location were not typical of herpes simplex, inoculation of a rabbit's cornea (Gruter's test) was done, and immunologic study of the inoculated rabbits was performed (i.e. demonstration of the appearance of the herpes neutralizing substance in the infected animals' blood serum; or if the animal developed an encephalitis, neutralization of the virus-containing brain tissue with a known herpes immune serum, the virus and immune serum being combined and inoculated intradermally in a susceptible rabbit).

The material[2] used in this study was prepared as follows: The entire brain of a rabbit dying of herpetic encephalitis[3] was macerated with 75 cc. of saline, containing 0.055 cc. of formalin and kept in the refrigerator for two days. Then 1.8

[2] This material was prepared under the supervision of Dr. M. Holden of the Department of Bacteriology, Columbia University.

[3] The E. L. 1 strain was used. This strain was originally isolated by Perdeau from a patient with epidemic encephalitis.

cc. of tricresol was added as a preservative to this mixture, and the heavy particles removed by centrifugation. The remaining material was then tested for bacterial sterility and for viable virus by injecting 0.4 cc. into a rabbit intracerebrally.

The following method was used in treating the patients. First, 0.1 cc. of a 1:10 saline dilution of the formolized herpes virus material was injected intradermally, with a saline control, and observed for any immediate wheal and pseudopod formation 15 minutes later. It is of interest to note that none of the patients treated showed any immediate reaction to this material. On the same day, the patient received 0.5 cc. of the formolized herpes virus material subcutaneously. Injections were given at weekly intervals alternately in the right and left arms. On the second week 1.0 cc. of the material was given subcutaneously. On the third week 1.5 cc. was given intramuscularly, and on subsequent weeks the dose was increased by 0.5 cc. until a maintenance dosage of 3.0 or 4.0 cc. given intramuscularly was reached. This level of dosage was maintained. Injections were continued until a total of 30 to 50 cc. of the formolized herpes virus was given.

Immediately following the injection the patients experienced, at the injection site, moderate pain lasting about five minutes. Some of the patients reported a slight stiffness in the extremity which had received the injection. This lasted about 24 hours. None of the patients had any systemic reaction following the injection.

From table I it can be observed that only one case of the fourteen patients observed failed to show any response to the formolized herpes virus therapy. It must also be noted that none of the fourteen cases treated showed a complete cessation of herpetic attacks. Three of the patients treated experienced only one outbreak in the year's treatment; and the remaining eleven all showed a marked reduction of the number of attacks. In many of the patients observed, previous to therapy, the duration of each outbreak had extended over a period of two or three weeks. This was due to the fact that these patients would have continuous fresh outbreaks of vesicles during this period instead of the usual initial outburst and then resolution ordinarily seen in herpes simplex. Of greatest importance is the marked decrease in the duration of the individual attacks following therapy. Most of these attacks would be distinctly abortive and be characterized by one, two or three isolated vesicles, instead of the usual cluster followed by more and more vesicles such as these patients had been experiencing. These isolated vesicles would dry in two to forty-eight hours and the crusts would be gone in two to four days. This phenomenon is rarely seen in untreated

TABLE I

		AGE	DURATION	LOCATION	INTERVAL	TOTAL AMOUNT OF F.H.V. RECEIVED	AVERAGE NUMBER OF ATTACKS PER YEAR		AVERAGE DURATION OF EACH ATTACK	
							Previous to therapy	Following therapy	Previous to therapy	Following therapy
						cc.			*days*	*days*
1	G. B.	59	1 year	Penis	4 weeks	30.5	13	3	14	4
2	G. L.	34	2 years	Penis	2–6 weeks	50.7	10	2	12	5
3	W. C.	31	More than 10 years	Penis	4–6 weeks	59.5	12	1	7	3
4	L. M.	36	2 years	Left hip	3–4 weeks with menses	73.8	15 (16 menses)	9 (16 menses)	11	3.4
5	N. J.	26	More than 20 years	Right cheek	4–6 weeks	30.7	10	1	10	4
6	W. G.	32	More than 20 years	Lips, cornea	4–6 weeks	13.9	10	3	10	2.3
7	M. K.	18	8 years	Nose and lips	2–4 weeks	30.3	20	21	8	8
8	N. K.	34	1 year	Right cheek	4–8 weeks	31.5	8	4	8	4
9	G. R.	20	20 years	Cornea, lips and tongue	Continuously every winter	33.3	Continuous	3	Continuous	6
10	N. F.	23	1 year	Upper lip	4 weeks with menses	5.2	13	3	10	5.3
11	L. E.	44	Many years	Upper lip	6–8 weeks	20.0	8	3	20	8
12	A. B.	24	Since childhood	Lips, chin and cheek	5–7 weeks	10.5	10	1	14	2
13	J. S.	29	4 years	Penis	1–8 weeks	36.0	8	7	8	4
14	T. H.	25	2 years	Penis	2–4 weeks	39.2	15	9	8	4

herpes simplex and would indicate a definite effect obtained from this material.

It is also of considerable interest that, at the onset of therapy, eight of the fifteen patients treated showed recurrences usually at the time that the next outbreak was to be expected. These outbreaks were markedly reduced in severity and duration, usually lasting only one or two days and can be characterized as abortive attacks. This observation also seems to indicate the efficacy of the formolized herpes material.

Five of the patients in this series have been observed for more than two years following the onset of the formolized herpes virus therapy. All of these cases, 1, 2, 3, 4 and 6, have shown the same decrease in frequency and duration of attacks in the second year following therapy as they did in the first year.

THE HERPES NEUTRALIZING SUBSTANCE

It was the purpose of this study to observe what changes occur in the neutralizing power of blood sera collected from individuals under various circumstances. Quantitative studies were made of the neutralizing titre of (1) sera taken both during and at various intervals between attacks, (2) sera from patients with recurrent herpes, before, during and after therapy with the formolized herpes virus material and (3) sera from individuals who, as far as they could recall, had never had herpes, as compared with sera collected from individuals who had histories of herpes.

The following method of determination of neutralizing titre was used: The E. L. 1 herpes strain was used. Since this strain is maintained by monthly passage through rabbit brains, it loses its dermatropic property. Rapid passage through rabbit testes, however, brings a return of the dermatropic characteristic. Once this "skin strain" was obtained, the next step was to dilute with saline the serum or the series of sera to be studied. In addition to the undiluted sera, the dilutions used were 1:10, 1:20, 1:40 and 1:80. The next step was to make an emulsion of the testicular virus-containing tissue, this emulsion consisting of one part testicular material and five parts of physiological saline. The emulsion was centrifuged and the supernatant material drawn off. Aliquot parts of this supernatant material was mixed with an equal amount of serum dilution and placed in the incubator at 37.0° centigrade for one hour. At the end of this time 0.2 cc. of each mixture was injected intradermally into the shaved skins of two rabbits. If the serum dilution used neutralized the virus, no reaction would appear on the rabbit's skin. However, if the serum dilution failed to neutralize,

a characteristic herpetic cluster would appear on the rabbit's skin. On each animal used, two controls were run, consisting of equal parts of (1) normal rabbit serum and virus, and (2) of known herpes-immune rabbit's serum and virus. Since rabbits vary in their reactivity to herpes virus, and since the virus itself varied in virulence from passage to passage, in order to make comparative studies, it was obviously necessary to run a complete series of sera on the same rabbit.

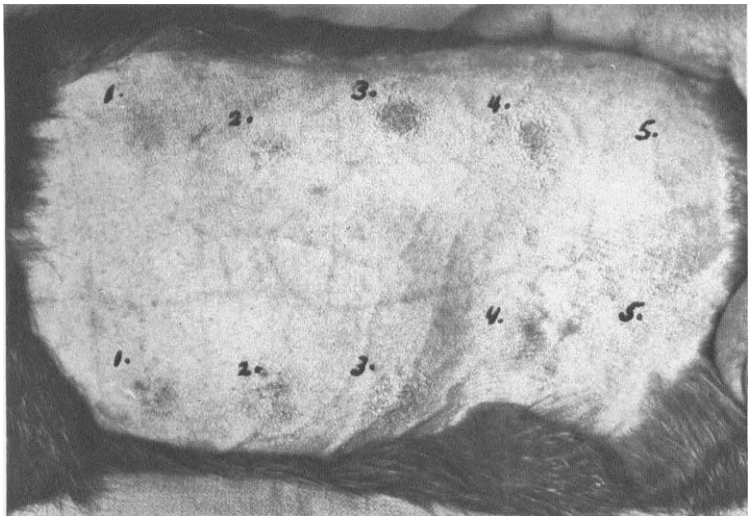

FIG. 1. NEUTRALIZATION TESTS ON RABBITS SKIN, ILLUSTRATING TYPE OF REACTION OBTAINED. INJECTION OF MIXTURE CONTAINING TESTICULAR HERPETIC VIRUS AND VARIOUS DILUTIONS OF PATIENT'S SERUM

Reading from left to right those reactions not covered by redundant hair.

Top row: (1) Unneutralized mixture, 0.1 cc. virus plus 0.1 cc. of 1:10 serum dilution. (2) Unneutralized mixture, 0.1 cc. virus plus 0.1 cc. of 1:20 serum dilution. (3) Unneutralized mixture, 0.1 cc. virus plus 0.1 cc. of 1:40 serum dilution. (4) Unneutralized mixture, 0.1 cc. virus plus 0.1 cc. of 1:80 serum dilution. (5) Neutralized mixture, 0.1 cc. virus plus 0.1 cc. of undiluted serum.

Lower row: (1) Unneutralized mixture, 0.1 cc. virus plus 0.1 cc. of 1:10 serum dilution. (2) Unneutralized mixture, 0.1 cc. virus plus 0.1 cc. of 1:20 serum dilution. (3) Unneutralized mixture, 0.1 cc. virus plus 0.1 cc. of 1:40 serum dilution. (4) Unneutralized mixture, 0.1 cc. virus plus 0.1 cc. of 1:80 serum dilution. (5) Neutralized mixture, 0.1 cc. virus plus 0.1 cc. of undiluted serum.

This made it unavoidable, in some instances to preserve serum for several months, until an entire series of blood specimens was collected from the same patient. Consequently all tubes containing serum were stoppered with a cotton plug and cork, and the ends of the tubes were then paraffinized. These tubes were kept in the refrigerator and any tube that showed bacterial contamination was discarded.

Phase 1. A series of blood sera collected from 5 individuals before, during,

following and between attacks of herpes simplex was tested to determine what change took place in the neutralizing titre of the serum at these various times.

Using the method as outlined above, every series of sera showed the same results as illustrated in table II.

Of the four remaining series studied, blood sera were collected at the following time in relation to the attacks of herpes.

Case A. S. 5/25/36 (7-day-old attack), 6/8/36 (21 days following the onset of the last attack and 102 days preceding a subsequent attack) and 9/18/36 (3-day-old attack).

Case T. M. 12/5/36 (1-day-old attack), 12/14/36 (10 days following onset of an attack), and 12/22/36 (18 days following an attack).

Case N. V. 7/23/36 (1-day-old attack), 7/29/36 (7 days following onset of an attack, and 12 days preceding a subsequent attack) and 8/10/36 (7 days after onset of an attack).

TABLE II

Studies on serum of patient A. V.

	SERUM DILUTION				
	*1:1	1:10	1:20	1:40	1:80
6/ 3/26 (attack 2 days old)	0	0	+	++	++
8/ 9/36 (attack 2½ days old)	0	0	+	++	+
7/14/36 (35 days from last attack)	0	0	+	++	++
7/21/36 (attack 1 day old)	0	0	++	++	++

* 0.1 cc. of each dilution of serum mixed and incubated for one hour with 0.1 cc. of 1:6 dilution of testicular herpes virus.

+ indicates failure of the serum to neutralize the virus and a consequent appearance of a lesion on the rabbit's skin. The number of +'s indicates the severity of the lesion.

0 indicates complete neutralization and no lesions.

Case J. R. 4/7/36 (1-day-old attack), 4/28/36 (15 days following onset of an attack) and 5/12/36 (39 days following an attack).

Three of these five series were repeated at three different times and the same general type of results was obtained.

From the above it can be noted that blood sera were collected on the first, second, third and seventh day of a herpes attack; on the 10th, 15th, 18th, 21st, 35th and 39th day following an attack; and on the 7th, 12th, and 102nd day previous to an attack. In no instance could a change in titre be detected in relation to the appearance of eruptions, and therefore the neutralizing titre cannot be considered a criterion of clinical immunity.

Phase 2. A series of blood sera was collected from four patients with recurrent herpes simplex, who had all received the formolized herpes virus therapy, and who had all experienced an apparent clinical improvement of their condition.

Using the same method of titrating the neutralizing substance as previously explained, in all four instances, the same type of results were obtained as illustrated in table III.

In all instances one or two samples were collected before and after the patient had received the formolized herpes virus, and were tested together with sera collected during the period of receiving this material.

Two of these four patients (W. C. and N. J.) had experienced only one attack in the interval during which blood samples were collected (approximately 7 and 9 months respectively), whereas

TABLE III

Patient W. C.

	SERUM DILUTION				
	*1:1	1:10	1:20	1:40	1:80
2/25/36	0	0	0	+	++
3/11/36	0	0	0	+	++
3/30/36	0	0	0	+	++
4/20/36	0	0	0	+	++
7/11/36	0	0	0	+	++
9/18/36	0	0	0	+	++

* 0.1 cc. of each dilution of serum mixed and incubated for one hour with 0.1 cc. of 1:6 dilution of testicular herpes virus. Treatment continued from 2/26/36 through June 15, 1936.

previous to this period W. C.'s and N. J.'s attacks were at a 4–6 week interval.

Of the remaining two, L. M. experienced seven attacks in the interval tested, and G. B. had four attacks, (during approximately 11 and 12 months respectively). However, in both of these cases, this was a marked decrease in the number of attacks as compared with a similar period immediately preceding.

It would seem therefore, that in spite of apparent clinical improvement during the period studied, there was no change in neutralizing titre in the blood serum of the four patients observed. This would also indicate that the neutralizing titre cannot be considered as an index of clinical immunity in herpes simplex.

Phase 3. Samples of blood were collected from six individuals who gave a history of never having had herpes, and from 22 individuals known to have suffered from herpes. The blood samples from the non-herpetics were collected from as reliable source as possible to obtain, 2 physicians, 2 technicians and 2 nurses. The bloods were titrated in the same way as in the previous two phases, namely against a known virus, and were inoculated intradermally on the same rabbit's skin.

Of the six blood sera obtained from the non-herpetics, three showed complete absence of neutralizing substances, and three showed a neutralizing titre of 1:20 (i.e. the 1:40 titre of serum was not adequate to prevent herpetic lesions from appearing on the rabbit's skin). All of the 22 sera obtained from the herpetics had this neutralizing property. Ten of these sera were tested undiluted. Of the remaining 12, six had a neutralizing titre of 1:20 and six of 1:40.

This would seem to imply, assuming the history of not having had herpes to be reliable, that three of these non-herpetics had never been infected by the herpes virus, and that the other three having been exposed, have shown an immune response without having an apparent lesion. However, it must be kept in mind that these individuals might have had hidden or unrecognizable lesions, or mouth lesions that they did not interpret as being herpetic.

It is also interesting to note that these six blood sera from individuals who have not had herpes were titrated concomitantly with a sample collected from a patient with recurrent herpes simplex. The latter also had the same neutralizing titre (1:20).

All of these observations would seem to indicate the inadequacy of the neutralizing titre as an index of clinical immunity.

It has been shown by many workers that the neutralizing substance appears in the guinea pig and rabbit only after exposure to the herpes virus (Flexner and Amoss (19), McKinley and Holden (20). However, this neutralizing property of the blood serum in humans cannot be considered as a criterion of clinical immunity.

It would seem that the neutralizing substance appears in the blood stream following exposure to the virus, but reaches an apparently stationary level which does not fluctuate, regardless of the clinical state in relation to attacks of herpes or clinical freedom. This would also seem to indicate that clinical immunity

to herpes simplex might not be a systemic phenomenon but rather a local one; and that other factors come into play, as to the nature of which we have no concept today.

As far as a more effective therapeutic approach to this problem is concerned, it is still possible that a more purified virus might be capable of raising the neutralizing titre of the blood to a level at which outbreaks will not occur.

SUMMARY

1. Fourteen patients with recurrent herpes simplex were treated with a formalin-treated herpes-infected-brain emulsion. Thirteen showed an increase in interval between attacks and a decrease of severity of the individual attacks. However, none of these patients experienced a complete remission. The recurrences following therapy were abortive and were characterized by the appearance of one, two, or three vesicles, which would dry in twenty-four to forty-eight hours, the crusts being gone in two to four days, a course not usually seen in untreated herpes simplex. This observation and the increase in interval noted would indicate the effectiveness of the formolized herpes virus material used.

2. Blood sera were collected from five patients with herpes simplex, before, during, following and between attacks and no change in neutralizing titre was noted at these different times.

3. Samples of blood collected from four patients who received formolized herpes virus were tested and showed no change in neutralizing titre in spite of clinical improvement experienced by these patients.

4. Blood samples from six individuals who never had herpes were studied for neutralizing titre. Three showed the presence of the neutralizing substance and three did not. In contrast to this, the sera collected from 22 patients who had had herpes, all possessed this neutralizing property.

5. The observations made seem to indicate that the presence or titre of neutralizing substance while the result of exposure to the virus is not a measure of clinical immunity.

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DISCUSSION

DR. SAMUEL M. PECK, *New York City:* This paper was most interesting, and I hope that it will lead us to an efficacious form of therapy in this rather stubborn condition. I would like to know in the light of the experiments just presented, how one would explain the effectiveness of the old method of treatment by the use of vaccine virus. I thought in some of my cases that my results were quite comparable to those presented by Dr. Frank. I would like to know if the blood serum of patients treated with ordinary vaccine virus contained an increased titer of antibodies in the blood serum, as he has shown with his formolized virus therapy. There might possibly be another explanation for these results by the theory of local tissue immunity according to the ideas of Besredka.

DR. GEORGE C. ANDREWS, *New York City:* I have been in touch with the work of Dr. Abshier in immunizing with smallpox virus against herpes simplex, and certain clinical features stand out as very interesting. Of course the results are not uniform. The period of freedom from attacks varies in different patients. Some patients are completely free for several years. Others have a series of six or eight weekly vaccinations and their period of freedom is only three or four months. However, in some cases, one does get splendid results. The number of vaccinations that should be given to any one case is always a matter of conjecture. I think that formerly six were recommended but now we feel that one should give ten or twelve. It seems that the more vaccinations you give, the better the results. Clinically often there is an immediate improvement in the lesion; that is, if a person comes in with a large herpes lesion, a patient who has had many attacks and each one usually takes several weeks to disappear, there is a definite improvement in the lesion a few days after the vaccination. It seems to be of immediate local benefit. In repeated vaccinations I have been impressed by the fact that there is nearly always a local reaction where you vaccinate, if it is carefully watched. Even though there is no vesicle or pustule formed, there will be a little local redness, or itching, coming on about the fifth day, and I believe there will be a change in the immunity in spite of the fact that there will not be a well marked local reaction.

DR. JOHN H. STOKES, *Philadelphia, Pa.:* I would like to ask whether any attempt was made to develop a local immunity at the expected site of occurrence of the lesion by injecting the formolized virus at the expected site; also, whether any study was made of the comparative effect of intradermal as compared to subcutaneous use of the formolized virus. Perhaps formolized virus cannot be injected intradermally. I do not know, and I should like to know.

DR. MARION B. SULZBERGER, *New York City:* As far as the practical aspects of this work are concerned, I think that I have now treated enough cases, some

with vaccination with the variola virus and others with the formolized herpes virus, to say that both methods have had signal successes and signal failures. However, the number of my cases is not sufficiently large to allow me to compare the relative practical merits of the two methods. Nevertheless, one can perhaps compare them from the theoretical viewpoint. One method uses the living variola virus, from the standpoint of non-specific effect of one living virus upon infection with another. This method has the disadvantage of being non-specific and the advantage of using a *living* virus. In the other method, that of Dr. Frank, one is employing the *specific* virus but it is non-living, it is dead. And so far there is no evidence to show that dead virus regularly confers immunity. In other words, we are not able, for example, to vaccinate against smallpox with non-living, dead smallpox virus. It would surely be a great advance if we could. There is another important theoretical and basic concept embodied in Dr. Frank's work. This is that the clinical immunity or susceptibility of the skin does not necessarily go parallel with the blood titre of immune bodies. I believe that this statement may apply not only to virus diseases but to others as well. In this connection I may state that we have adequate evidence both from the literature and our own experiments that the immunity or susceptibility of the skin to staphylococcus toxin does not necessarily run parallel with the titre of anti-toxic units in the blood serum. (Sulzberger and Rubin: Discussion to Tannenbaum, Joyner, Speed and Bremer, J. Allergy, **9**: 241, 1938.)

DR. D. C. SMITH, *University of Virginia:* The development of the ultra centrifuge and its use in the separation and purification of virus proteins will undoubtedly lead to improved methods for this study. It may well be that a virus vaccine prepared with the addition of formalin is ineffective because of chemical changes produced.

DR. GEORGE S. WILLIAMSON, *Ottawa, Canada:* I would like to ask any of the members, or Dr. Frank, if they have attempted to use blister fluid for local inoculations, and if so, with what result? I have employed this procedure in a small group of patients with apparently encouraging results, but was unable to arrive at definite conclusions from this small group.

DR. SAMUEL M. KAUFMAN, *New York City:* I would like to know how Dr. Frank interprets the improvement in cases of herpes simplex by the treatment of fractional doses of roentgen rays and the diminished recurrences.

DR. GEORGE SCHWARTZ, *Boston, Mass.:* I would like to ask Dr. Frank if he has used controls with other non-specific substances, and whether, if he has, there has occurred an increase or decrease in sensitivity. I wonder if the effect here is specific or non-specific?

DR. FRED D. WEIDMAN, *Philadelphia, Pa.:* I noticed in the table one outstanding instance, a patient aged eighteen where the therapy was not effective. I would like to ask if there were any data peculiar to that case which might throw light on that situation?

DR. I. J. ARNSSON, *Buffalo, New York:* In using smallpox vaccine, the scratch method is less effective than the intradermal.

DR. SAMUEL B. FRANK, *New York City:* I want to thank the discussers for their contributions. It is believed today that there is no relationship between the vaccinia and herpes virus. However, clinically we do see therapeutic results. In discussion with others, several have made the observation that they get their best clinical effect when a take is obtained with vaccinia. Perhaps the vaccinia reaction makes the skin less reactive to an attack of herpes, as seen in the relationship of measles to the Dick test.

In answer to Dr. Stokes, I made no attempt to inject the material at the site where the recurrence was usually found. I was mainly interested in seeing what large amounts of the material would do, rather than in the intradermal inoculation.

I have tried inoculation of blister fluid from a herpetic lesion to another area several times and have not obtained a successful take. In contrast, I've been able to get a take on myself with no difficulty. However, I rarely have herpes and did not have one at the time. Perhaps the failure to get homologous takes may be explained by a skin immunity that makes it resistant to an inoculation during an attack.

I have not used any other protein or foreign substance in treating patients.

It is possible that the clinical effect of x-ray preventing recurrences of herpes in some cases may be explained by some alteration of the virus, assuming the virus remains latent in that area, or a change in the tissues making it unfavorable to the virus.

In answer to Dr. Weidman, I have no explanation for the failure to get a clinical effect in the patient he refers to.