PART VII

Immunity

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IMMUNITY

The theoretical and practical problems of immunity in syphilis have produced a bewildering array of experimental and clinical reports, have stimulated extensive research programs, and have given rise to a vast volume of literature which has been well summarized in English first by Chesney in 1926 (49) and more recently by Urbach and Beerman (66). Rather than to attempt an extensive review of the problem here the discussion will concern itself only with those aspects of immunity and allergy which are relevant to the experimental procedures.

Immunity of the body to a pathogen can be considered to reside at several levels. The first is the surface of skin and the epithelial lining of the digestive, genito-urinary, respiratory systems and the conjunctivae. The second level of immunity can be considered to reside in the tissues. The third may be considered to be humoral, or blood-and lymph-borne.

Finally, immunity of the body to an organism must be considered in relation to the organism and even the strain of organisms causing the infection. Much has been written about the difference in invasiveness of strains of spirochetes from different areas of the world and about the possible differences brought about in the organisms by virtue of passage through a susceptible animal.

In the course of our studies which were only secondarily designed to study such theoretical problems, numerous opportunities arose to investigate more closely certain of the problems relating to the resistance of the body to infection and to the host-parasite relation. These factors will be discussed in order in the following section.

Skin and Mucous Membrane in Resistance to Infection

Review of the rates of infection through intact mucous membrane, through abraded mucous membrane, and by introduction through the skin as discussed in the Prophylaxis Section reveals a striking gradient. Only when the organism is introduced through the surface, or when the surface is injured, is infection almost certain to take place. This observation only adds experimental confirmation of the commonplace observation that a pathogen or its toxic products must penetrate the covering layer of skin or mucous membrane in order to produce harm to the tissue. While much has been written as to the mechanism of resistance of the skin to bacterial penetration, and while abundant evidence exists to show the constant presence on skin surfaces and in body cavities of potential pathogens, we still have no conclusive proof of what various factors are involved. Whether protection depends merely upon mechanical inability of the pathogen to penetrate the skin or mucous membrane covering or whether secretions, or even tissue itself contains factors which are lethal to the bacterial life is not definitely known. It is not, however, our purpose here to review all factors concerned.

But from our own experiences in comparing rate of infection through the intact mucous membrane of the penis as compared with that following scarification it would seem that the mere existance of the mechanical barrier of the intact epithelium is an extremely important factor in prevention of establishment of the infection within the body.

No experiments were performed to attempt to measure differences in resistance between intact skin and mucous membrane. However, in inoculation techniques involving the penis there was almost invariable contamination of the external skin surface of the prepuce or of the skin of the shaft of the penis in those with short foreskins. In no instance did we observe development of a lesion on the skin surface of penis or prepuce unless, as in several instances, injection had been made into skin rather than mucous membrane. This experimental observation is in harmony with the clinical finding that in naturally acquired syphilis it is uncommon to find a chancre on the shaft of the penis or in the pubic area or on the scrotum in spite of the contamination which must take place during intercourse, especially when the chancre of the female is located on the labia, the fourchette, or at the introitus.

We further noted proof of resistance of skin in multiple-pressure inoculation. The drop of inoculum spread from the area of multiple pressure as soon as the patient permitted the arm to fall to the side upon completion of the procedure. No attempt was made to wipe off the excess. However, in no instance was there formation of multiple skin chancres such as would be expected if infection were to take place through the intact skin as well as through the traumatized skin.

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The intact mucous membrane of the gastro-intestinal tract seems also to offer a barrier to penetration of T. pallidum as shown by low rates in the attempts to infect by ingestion of T. pallidum. All of these infections were asymptomatic, so that no speculation can be made about the site of penetration. The low rate of infection parallels that found in attempts to transmit enteric infections experimentally by ingestion and suggests further need for study on the mechanism of resistance to enteric routes of infection. What are the roles of tissue secretions in resistance, for instance?

At what level penetration of the lining took place cannot be stated, but no lesions were noted to develop in the oral cavity or the oro-pharynx. The relatively low rate of infection of 2 in 6, Exp. 0604, when related to the massive dose of organisms taken, 10^7, suggests that this system, also, has a relatively high degree of resistance to infection.

The importance of the integumental barrier to infection became apparent with succeeding experiments in which by various means the organism was introduced under the surface or applied to the surface whose continuity had been interrupted mechanically by abrasion or multiple pressure. By whatever means the organisms gained access through the cutis or mucosa, the previously non-syphilitic individual showed almost universal susceptibility to infection so long as the total dosage of organisms was above a minimum critical level which appears to be on the order of 1,000, as shown by experiments 1302, 1303, and 1304 in which graded doses of inocula were used.

It would thus appear that the intact skin offers an effective barrier to passage of the T. pallidum, just as does the intact mucous membrane. In our experimental studies, injury to that integument, by mechanical means in this group, resulted in almost complete abolition of resistance to infection in the individual exposed for the first time. For in contrast to a rate of infection of 17.9 percent (7 of 39) found in those exposed to application to the intact mucous membrane of the penis, scarification of the penile mucous membrane with local application gave a rate of 91.6 percent or 76 of 83 patients. Then each technic involving penetration of the skin, whether by multiple pressure or injection intracutaneously gave a rate of 96.2 percent (25 of 26) and 96.8 percent (150 of 155) respectively.

The significance of interruption of continuity of the integument in relation to transmission and acquisition of infection through intercourse can be related to both experimental and natural factors. As mentioned earlier, observations of large groups of men immediately after sexual contact with prostitutes revealed in most of them changes in the penile mucous membrane ranging from simple reddening, capillary enlargement, through evidence of abrasion to frank bleeding in some, particularly from the frenum. This suggests that the trauma of sexual intercourse is probably responsible for breaks, minute or gross, in the male genital mucous membrane which then renders him highly susceptible to infection if treponemal containing fluid with sufficient quantity of organisms comes into contact with the breaks.

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TABLE 25

LISTING OF PATIENTS INFECTED AFTER ONE OR MORE UNSUCCESSFUL ATTEMPTS TO INFECT

NEGATIVE PRIOR TO EXPERIMENTATION

Patient	Sex	Previous Exposures to Infectious Material Method of Inoculation	Infectious Exposure Method of Inoculation
118	M	Local app.	Scar.
119	M	Local app.	Scar.
122	M	Local app.	Scar.
123	M	Local app.	Scar.
126	M	Local app.	Scar.
141	M	Ingestion	Intracut.
143	M	Ingestion	Scar.
156	M	Local app.	Scar.
158	M	Local app.	Scar.
182	M	Local app.	Scar.
183	M	Local app.	Scar.
184	M	Local app.	Scar.
185	M	Local app.	Scar.
190	M	Local app.	Scar.
193	M	Local app.	Intracut.
194	M	Local app.	Intracut.
211	M	Scar.	Intracut.
234	F	Intracut.	Intracut.

FALSE POSITIVE PRIOR TO EXPERIMENTATION

Patient	Sex	Previous Exposures to Infectious Material Method of Inoculation	Infectious Exposure Method of Inoculation
120	M	Local app.	Scar.
124	M	Local app.	Scar.
128	M	Local app.	Scar.
130	M	Local app.	Scar.
188	M	Local app.	Scar.
189	M	Local app.	Scar.
192	M	Local app.	Intracut.
199	M	Local app.	Scar.
200	M	Local app.	Scar.
201	M	Local app.	Intracut.
218	M	Local app.	Intracut.
105	F	Mult. Press	Mult. Press

Further to speculate on the significance of these findings it is necessary to recall the studies showing the relationship of circumcision to acquisition of venereal disease. It has been demonstrated that the rate of infection, not only of syphilis and chancroid, but also of gonorrhea, is lower among those circumcised than those uncircumcised.(51) Following circumcision there are changes in the mucosal covering of the glans penis and remaining foreskin resulting in thickening, cornification, and presumably increased resistance to disruptive forces which would suggest that the trauma of intercourse would result in less breaks in continuity and would not predispose to permitting penetration of pathogens such as T. pallidum.

Once the organism has gained access to the body several factors must be considered—the speed of spread from the site of introduction, the rapidity of reproduction, and the rate of development of lesion or serologic evidence of infection as related to the immune status of the body, prophylactic procedures, etc.

Previous studies have been done on the speed of dissemination of the organism in the rabbit following introduction below the surface of the skin. Lymph-node transfer studies have shown that within 5 minutes the organism has passed to the regional lymph nodes (52). Experiments in testing locally applied prophylactic agents in the rabbit have shown that the sooner the prophylactic agent for local use is applied after application of the virulent emulsion, the better the protection given. To be most effective treatment must be given locally within about 2 hours, after which efficiency rapidly decreases (34). The discrepancy in the two different time periods involved in generalization of the organism as shown experimentally suggested the need for further study in man.

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In local application to the abraded mucous membrane application lasted two hours. With that length of application, the rate of infection was 83 in 96 or 91.6 percent. However when prophylaxis was applied locally at the end of a 2 hour exposure, protection was complete in 29 or 30 patients. However, prophylaxis of the same type applied 4 hours after final inoculation with a 2 hour application, permitted the development of infection in 3 of 9 patients. These findings suggest that penetration of the organism, even though abrasion is relatively slow, so that it is localized and susceptable to the action of locally applied treponemacidal agents for at least 2 hours and that even 4 hours after termination of a 2 hour period of application, the spread of organism from the site of application is of a low order of magnitude. In view of the demonstration that 10 organisms can cause infection and about 10^4 or more must be introduced in order to uniformly produce infection, it appears that if local prophylactic agents are capable of destroying the majority of organisms, the occasional escape of small numbers may not be important in that the defense mechanisms of the body may be sufficient to cope with them. However, the study again demonstrates the necessity for application of the active agents before the organisms have escaped from the zone of influence of such agents.

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Tissue Immunity

Techniques introducing organisms directly into the blood stream or into spinal fluid invariably produced infection. Significant reduction in rates of infection when considered with respect to numbers of organisms in the inoculum did not begin to appear until the inoculum dropped below the level of 10^4 organisms. Yet there was failure to secure infection following techniques of intracutaneous, scarification or multiple pressure inoculation in individuals who had never before, according to their history, had syphilis, and who had neither clinical nor serologic evidence of previous infection.

Whenever possible the individual not infected as a control was reinoculated by the same technic or different if indicated, Table 25. In each such instance when the nonsyphilitic individual was reexposed to inoculation, infection took place. Thus, the immunity previously shown and not apparently based on previous infection was apparently relative to factors in the individual and inoculum or method at the time. It did not indicate absolute tissue immunity.

There seems to be only one explanation which can be given for this finding of relative immunity. It seems, as has been postulated and shown both experimentally in other attempts at experimental infection and in the study of natural epidemiology of disease, that there is non-specific tissue resistance to penetration by and growth of pathogenic organisms. Our experience in which about 1 in 10 of those exposed revealed resistance to establishment of disease suggests the possible extent of the degree of non-specific resistance to syphilis within the general population. This finding is of interest epidemiologically and experimentally. For to our knowledge in experimental inoculation of the gonococcus, the virus of hepatitis, or the malaria parasite, there has never been found, under physiologic conditions uninfluenced by attempts to overcome resistance by various means, absolute susceptibility to the pathogen. In our experience it would appear that this resistance may be relative rather than absolute in the case of syphilis. For reference to Table 25 reveals the outcome of reexposure of individuals who had failed to acquire infection following first exposure to inoculating technics not followed by prophylactic procedures. It will be seen that the majority of those resistant to infection were resistant to infection by a method having a very low rate of infection--local application. In each instance the patient later developed infection when subjected to a method which traumatized the skin or mucous membrane so as to insure the entrance of the organism to the body.

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However, in a few instances the individual was not infected by methods of multiple pressure, scarification and local application, or by intracutaneous injection. Some of these individuals considered here had evidence of previous infection, either serologically or by physical findings. Five individuals resistant to infection following scarification and local application were failures from the same experimental run. It is our feeling that there may have been factors in the inoculum itself which was responsible for these failures being concentrated on this particular day even though some patients given prophylaxis on this day showed failure of prophylactic protection. (Exp. 1204, 1205, 1206)

Those showing failure following intracutaneous injection were not confined to any one particular day or experiment, so that they seem to represent failures which must be dependent upon factors in the individual or experimenter rather than in the technic or inoculum. But by chance alone it might be possible through the workings of the same factors, for most of the failures of the method of scarification to have occurred on one particular day.

In any event, the fact that these same individuals could be successfully inoculated at a later date, with either identical or different technics suggests that immunity such as seen here is relative rather than absolute. It would seem as though there are factors within the individual which are variable so that at one time he can resist establishment of an infection to which he will be susceptible at another time. These factors are apparently, in this group, unrelated to previous experience with the pathogen and are rather expressions of what may be

considered as 'natural', or 'non-specific' resistance of the body to pathogenic agents.

In our clinical studies to determine risk of infection acquired sexually, two prostitutes were inoculated by intra-cervical injection of rabbit testicular syphilomata. Neither developed a chancre or secondary eruption, but both developed serologic evidence of disease, later becoming negative after specific therapy. Twelve volunteers had single exposures to one or the other, all without use of condom or pre or post coitus prophylaxis. In a 4-month period of observation none developed clinical evidence of infection. Serologic data was not complete and could not be obtained on all due to patient reluctance to permit repeated bleedings. But in view of other evidence found in this study, it is possible to state with a high degree of certainty that at the most only one or two might have had asymptomatic infection. Thus the risk of infection following single contact with an asymptomatic female with early syphilis is slight.

In our own experience in epidemiologic studies on transmission, the large group which can be used to compare with controlled studies on contacts of individuals known to have infectious syphilis, was that reported from India (53). There in an isolated, relatively closed community were found sexual and marriage habits which provided for rather free sexual contact. The environment had not been altered by the introduction of specific therapeutic agents for treatment in large amounts, and there had been no organized effort to control syphilis.

TABLE 26

Interpolated Values of Time to Clinical Evidence of Patients Inoculated at Two Sites by the Same Method, Strain, and Inoculum Size

Experiment Number	Method	Inoculum Size	Days to Clinical Evidence by Site Arm	Penis
0503	Intracutaneous	10^6	2.5	7.0
			7.0	19.5
			7.0	2.5
			12.5	24.0
			2.5	6.0
			7.0	8.5
0601	Intracutaneous	10^6	4.5	17.5
			4.5	14.0
			4.5	10.5
0703	Intracutaneous	10^5	7.0	10.5
			14.0	7.0
			2.5	7.0
			2.5	29.5
			10.5	22.5
			2.5	10.5
			10.5	10.5
0906	Multiple Pressure	10^5	24.0	24.0
			21.5	——
			40.0	19.0

It was found that about half of the adult population was infected with syphilis. Review of the marriage and sexual history of these uninfected revealed that in most cases the individual had had multiple sexual partners, and was, at the time of study, not infrequently living with an individual having either clinical or serologic evidence of syphilis. The field study quoted, revealed findings in harmony with those reported in clinical work in which only about half of those exposed to infectious syphilis are infected (29,30). The experimental studies here reported validate the hypotheses drawn from the clinical experience in showing unequivocal evidence of non-specific resistance to infection with syphilis. It is probable, on the basis of our experiments, that the defense mechanisms reside in the intact skin and mucous membrane surfaces, in integumentary tissues, as well as in blood and lymphatic systems.

Attempts to Overcome Immunity

In an attempt to overcome possible body immunity and in view of evidence obtained in work with other organisms that mucin has the ability to reduce the defense mechanisms of the body it was used early in the course of studies as a component of the menstrum (31). However, following experimentation there was no evidence to indicate that when applied locally and containing T. pallidum, mucin preparations had any effect in lowering the resistance of the mucous membrane to penetration by T. pallidum.

Skin and Mucous Membrane Compared

There is no significant difference in rate of infection through skin or mucous membrane if the mucous membrane is injured by injection or scarification. However, as noted in the clinical section, Table 17, there is a significantly greater rate of asymptomatic infections found following infection through the mucous membrane and not related to prophylactic failure.

It is also found that there seems to be prolongation of incubation time following infection through the non-intact mucous membrane. The factor of incubation time was carefully studied in a group of patients given simultaneously equal quantities of inoculum by injection into the mucous membrane of the penis and intracutaneously into the skin of the forearm. The incubation periods in the two sites are shown in Table 26. There it will be seen that with but few exceptions the cutaneous lesion developed before the mucous membrane lesion. In some individuals, though the incubation periods were about equal, in several the opposite was found, and in one of the group no mucous membrane lesion at all developed.

In consideration of the factors related to length of incubation period it was found that there is a general direct relationship between size of inoculum, and incubation period; the smaller the inoculum the longer the incubation period. Consideration of this finding as related to differences in incubation period to lesion syphilis in skin and mucous membrane of the same individual given equal inoculum in each tissue by identical methods suggests the possible working of several factors. Slowness of reaction to an infectious agent, in development of what has been considered by some as the allergic state which is a factor in development of the chancre, may represent fundamental differences in the anatomic structure of skin and mucous membrane, differences in blood and lymph supply, and in the reticulo-endothelial and lymphoid components. It may well be that the mucous membrane does not have the same allergic potential as skin.

There is another possibility to consider. There appears to be different potentialities of tissue resistance to a given organism related to the location of the tissue and possibly resulting from differences in skin secretion, pH, and other facts not well understood (31, pages 1019-27). If the genital tissues had a higher degree of resistance to T. pallidum than skin, there should be destruction of the inoculum in situ. Extensive destruction, reducing the effective size of the inoculum might then be responsible for the significant lengthening of incubation period. This factor might even be called into consideration with respect to the asymptomatic state. If there

were rapid and extensive destruction of T. pallidum *in situ* the net result might be considered to be similar to that observed following intravenous inoculation without contamination, Exp. 1010. The infection would then result from invasion of the blood or lymphatic system by the few organisms escaping from the site of inoculation; with complete destruction of all organisms at the site of inoculation, therefore there would be no localized collection of organisms to give rise later to the phenomenon of a chancre which is apparently related to the persistance of organisms at the portal of entry into the body. Thus, the infection would be asymptomatic or attended by lymph node enlargement and perhaps development of secondary lesions.

Source of Organisms in Relation to Immunity

There has been some evidence adduced from inoculation of rabbits with human-strain T. pallidum injected for the first time into rabbits, that there may be factors in both host and parasite which necessitate adaptations in T. pallidum before it will grow satisfactorily in the animal. This suggests that there may be immune factors in the animal which must be overcome by adaptation of the parasite before the parasite develops the capacity to produce regularly and uniformly a certain clinical syndrome in the animal or conversely, in man, when the rabbit is the source of the inoculum. Our experience, as summarized in Table 24, revealed to us no difference in capacity to cause infection or in type and nature of infection produced in man regardless of the source of T. pallidum with which he was challenged. The immunity, or lack of immunity of the human, was unrelated to the source of the organism.

Our findings further suggest that if any changes have taken place in T. pallidum adapted to growth in the rabbit they are of such a nature as not to be evident by the type experiment which we did and as not to modify the clinical or serologic course of infection produced in man. These findings suggest a restudy of the entire question of adaptation of an organism strain from man to animal.

Reinfection

At the time this study was conceived and during its operation there was serious doubt expressed by many of the leading syphilologists, (55) as to the ability of penicillin to cure syphilis. There were at that time many unanswered questions regarding immunity and reinfection in syphilis, (50), and there was much controversy regarding criterias for determination of whether or not diagnosis of reinfection could be made with any degree of certainty. This latter fact was vital in determination of whether or not penicillin failures being seen were reinfections or relapses, and the syphilologists insisted upon observation of extremely rigid, and what seemed to us as unrealistic criterias for calling a failure to penicillin therapy a reinfection (56). This study provided an ideal opportunity for the VDRL to answer to its own satisfaction, at least, some of the problems in this field.

The first question to be answered was whether or not reinfection could take place.

In the entire body of experiments designed to answer these questions no attempt was made to study quantitative relationships between ability to reinfect and the dosage required to overcome resistance. There were several reasons for this. However, we were primarily determined to find whether or not infection could take place. As time went on and as we observed differences in incubation period, rates of infection as related to size of inoculum, etc., it appeared that an exhaustive study of those factors was out of the realm of possibility at that time. For time allowed for experimentation was limited, as were funds. The sources of clinical material were not limited, as were funds. The sources of clinical material were not limitless, and, most important, it early appeared to us, in view of the close correlation between our human experimental findings and those observed in studying the same question in the rabbit, that many of those factors involving quantitative measurements in study of resistance could better be worked out in the rabbit. For by such experimental work sufficiently large numbers of experimental subjects would be available to provide statistically valid answers. Thus we confined our studies to qualitative rather than quantitative experiments, using in general, for reinoculation experiments dosages of inoculum of approximately the same magnitude as used in the original experiments. In selected experiments when we were attempting to overcome resistance in patients with latent or late syphilis the dose of organisms was very much larger than that usually employed.

The first question to be answered was whether or not reinfection could take place following treatment for primary or secondary syphilis regardless of the age of the original infection at time of treatment and regardless of serologic status at time of reinoculation. The findings were that to the same extent that the first infection could be established by intracutaneous inoculation it was possible to reinfect a group of patients treated with an adequate dose of penicillin, 4,250,000 U. or more given so as to maintain a blood level for one week (see section on treatment).

The patients upon reinoculation revealed the same pattern of development of lesion as exhibited following the initial infection, although there were, as will be discussed, some significant differences in type of lesion and in time sequence. Further studies revealed that following treatment for primary or secondary syphilis it was uniformly possible to reinfect the individual regardless of the duration of the initial infection regardless of whether he was challenged a week or a year after treatment, and regardless of the serologic titer at the time of reinoculation.

Proof of the fact that the lesions represented reinfection rather than relapse is found from examination of the patients not reexposed yet treated with the same dosage of penicillin. As stated in the section on treatment, with the dosage schedules standard for the experimental groups, only 1 individual out of 255 treated for the original infection and not challenged by reinoculation required re-treatment on unequivocal grounds, and this patient showed clinical evidence of his failure. There were 62 experimentally infected for the second time and 9 experimentally infected for the third time. All of these showed clinical or serologic evidence compatible with the interpretation of reinfection. The underlined statement in the last sentence was made deliberately, for that evidence is the same as could be considered as representing relapse were not the relationship known between the challenge of the individual by reinoculation and the development of the clinical and serologic findings. In other words, there is no clinical or serologic means by which a reinfection can be unequivocally distinguished from a relapse. There are time differentials in clinical sequence and there are some clinical characteristics more common to reinfection than to first infection, yet of the nature of lesions described to occur in the relapsing type of secondary syphilis. However, the variety of possible lesions, combinations of lesions, and time sequences in development of clinical and serologic evidence of infection is so great that it appears to us impossible to make the unequivocal decision as to relapse or reinfection in the individual case on any grounds other than epidemiologic. Consideration of the exposure history, and the adequacy of previous treatment must be taken in order to arrive at a decision in each case.

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As has been pointed out in the section on controls and in the preceding section, adequate controls of heat-killed inoculum were used to provide assurance that the lesions observed were truly those of reinfection rather than those of skin-sensitivity to the protein, and darkfield conformation was likewise obtained often enough to assure the specificity of the lesions.

Not only did we determine that reinfection could take place when the inoculum was injected at a different site and by a different method the second time, but also we determined that reinfection could take place when the second injection was made into the scar resulting from the first infection. In view of skin pigmentary changes it was a simple matter to be sure that the inoculation was made exactly in the same spot as the original. This, too, can be summarized by stating that reinfection took place in each instance of the 10 injections onto the scars of the first infection. This is in contrast to experimental work summarized by Beerman (50 which suggests a measure of immunity in the previously-infected site. It is quite possible that the size of inoculum used was so large as to overcome low grade immunity if any did exist.

There was, however, evidence of allergy in the sense used by Urbach and Beerman (50). If it is assumed that allergy is an expression of immunity and if it is assumed that the patient challenged by reinfection will show evidence of that allergy by clinical differences in the lesion, in the pathogenesis of the infection, and in time-relationships, then our findings can be explained.

We have previously discussed the matter of asymptomatic infection and the various factors involved. We have drawn a hypothesis based upon local tissue immunity, in the case of asymptomatic infections following mucous membrane inoculation. If, in the absence of prophylactic failures, local tissue resistance is a factor in increasing the rate of asymptomatic infections, the following findings can not be explained. For it is found here that the rate of asymptomatic infection is less in those previously infected than those infected the first time as shown in Table 27.

Table 27

Proportion of Asymptomatics Found Among Negative Patients Previously Uninfected and Those Previously Infected

Previous Infections	Total Infections	Asymptomatic	
		Number	Percent
No Previous Infections	298	25	8.3
Previously Infected	73	5	6.8

(Infections produced by the technics of ingestion and intravenous inoculation have been omitted).

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It would appear that a factor other than simple resistance to infection were operative here. If there is alteration of the immune mechanism of the skin as a result of the first infection resulting in incomplete immunity it is possible that if the infection cannot be suppressed, the normal course of multiplication of T. pallidum in the body will be reflected by an increased propensity to a local-allergic reaction at the site of inoculation. Hence the patient reinfected may have a greater tendency to development of a lesion at the site as a result of tissue sensitization. In other words, the body is sensitized to the protein so that the factors of sensitivity which are supposed to be operative in the original development of the chancre (50) are enhanced in reinfection by sensitization resulting from the first infection.

Consideration of tissue sensitization leads into a discussion of the phenomenon which we named "local secondary like reactions." This term was applied to a syndrome in which, after the development of the primary lesion, and apparently forming an integral part of the lesion of inoculation there appeared surrounding the site of inoculation and immediately adjacent to it the entity which is described in the following paragraph. This local reaction was confined to the vicinity of the inoculation, but at times there was noted the subsequent development of a generalized secondary reaction in the patients showing this localized reaction. However, this generalized secondary reaction followed after

the development of the primary lesion of inoculation and the localized secondary-like reaction. Although the coloration of the primary and associated local-secondary-like lesions was usually more intense than that of the first infection, a number of the patients with previous history or clinical or serologic evidence of syphilis, tended to show greater induration and deeper coloration, particularly of a deep violaceous hue, than those showing the same local secondary like reaction not related, to the best of our knowledge, to previous infection. A general description of the lesion follows:

Local Secondary-Like Reaction

At the site of injection the papule formed as was expected and underwent the usual course but somewhat later in the course of development, the patients showed in the skin surrounding the site of inoculation, a pink macular, papular or maculo-papular eruption identical with the lesions of classical secondary syphilis but sharply localized to the area surrounding the site of inoculation. In some patients, these manifestations showed spontaneous regression, while in all, treatment resulted in disappearances of lesions. It is of interest to note that at the onset of penicillin therapy, these lesions usually showed increase in size and depth of coloration exactly as noted with classical secondary syphilis when penicillin treatment is instituted. The surrounding skin not infrequently showed edema and mild pruritis as an accompaniment of the onset of penicillin therapy. There was usually no pigmentation of the sites of local secondary-like reactions after healing in contrast to pigmentation often remaining at the injection site unless the local secondary-like reaction was particularly extensive,

deeply pigmented, and indurated before treatment was started.

In Table 28 the distribution of the 32 patients having this type of reaction is given with respect to sex, residence, and previous infections. In addition to the various breakdowns given in the table it might also be added that all of these were inoculated in the skin by the intracutaneous method and none represented prophylaxis failure.

The "local secondary-like reaction" was observed in 13 patients out of 142 inoculated for the first time. Of these, 11 were found to occur among 28 so inoculated at the penitentiary. Of those in whom this reaction consisted of a reinfection, 19 of 21 patients were observed to have this type of reaction. When the rates of secondary-like reactions resulting from first infections are compared to those from reinfection the differences are found to be significant.

The four male patients from the penitentiary showing a secondary like reaction as a result of reinfection were from a group of 18 selected patients considered to be latent cases at the time of selection. Of these 18 patients, 7 received penicillin therapy prior to inoculation while the remaining 11 received no treatment. (These groups are discussed in the chronology, experiments 1402 and 1403 respectively). Of a total of 5 infections occurring in each experiment 2 from each were noted to have secondary-like infections.

TABLE 28

Local Secondary Like Reactions of Patients Inoculated Intracutaneously Into the Skin and Without Prophylactic Protection

	Male		Female	
	Penitentiary	Asylum	Asylum	Total
No Previous Infection	11/28 = 39.3%	0/44 = 0.0%	2/70 = 2.8%	13/142 = 9.2%
Reinfection	4/10 = 40.0%	7/30 = 23.3%	8/41 = 19.5%	19/81 = 23.4%
Total	15/38 = 39.5%	7/74 = 9.4%	10/111 = 9.0%	32/223 = 14.3%

It is difficult to explain the significance of the fact that 11 of the 13 patients showing this secondary-like reaction as a result of their first infection were males in the penitentiary group. However, the prisoners as a group appeared to the authors to be better nourished, to have better skin nutrition as manifested by more subcutaneous tissue, better turgor, and a better sheen than did the group at the asylum. It has been observed that in conditions of extreme cachexia or in patients in advanced stages of disease such as tuberculosis, the skin shows an anergy. It may be that the differences here mentioned may result from a similar set of circumstances, perhaps a relative anergy of the skin in the poorly nourished group.

The explanation that the reaction might be caused as an allergic reaction to exposure to rabbit tissue for a second time can be disproven by the following facts:

1. So far as is known, none of the patients inoculated for the first time and showing the reaction had ever been exposed to the injection of rabbit tissue. In view of the lack of reaction of a similar group at the penitentiary given emulsions of rabbit tissue and showing no similar reaction to the first inoculation, the possibility that the group showing this reaction was reacting to a rabbit protein and not to a spirochete is highly unlikely.

2. A group of 15 patients at the asylum were inoculated intracutaneously with a rabbit testicle, serum emulsion and protected by penicillin prophylaxis. After 12 or more days observation they were reinoculated—4 with human strain spirochetes in human serum tissue emulsion and 11 with rabbit strain spirochetes in rabbit testicle serum emulsion. None of these patients developed a "local secondary-like reaction" in spite of the fact that all had been exposed to living spirochetes which were presumably destroyed by the penicillin resulting in exposure of the body to products of spirochetal disintegration (the assumption that the body was exposed to living spirochetes is a valid one, as all controls were infected). Eleven of the group were exposed for the second time to rabbit protein without showing any evidence of sensitivity.

From these two reasons it is thus clearly evident that the reaction under discussion is not a response of the body to rabbit protein.

The duration of the first infection is of no apparent significance in the pathogenesis of the "local secondary-like reaction" for the average duration of the first infection before treatment in the 15 patients at the asylum was 47 days against 51 days in the 71 patients reinfected under the same conditions but not developing the reaction.

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In the 191 patients not developing a secondary-like reaction the average duration of infection before treatment was 80 days. Of the 32 developing the reaction the average incubation period to time of appearance of the lesion was 8 days. Thus it is evident that those not developing this reaction were allowed to retain the infection long enough for the reaction to develop.

TABLE 29

Listing of 32 Patients Showing Secondary - Like Reactions

Patient Number	Experiment Number (old)	Sex	Previously Infected	Residence
018	0706	Male	Yes	Penitentiary
024	0704	Male	No	Penitentiary
052	0705	Male	No	Penitentiary
060	0707	Male	Yes	Penitentiary
061	0705	Male	No	Penitentiary
062	0706	Male	Yes	Penitentiary
064	0707	Male	Yes	Penitentiary
066	0705	Male	No	Penitentiary
073	0705	Male	No	Penitentiary
074	0705	Male	No	Penitentiary
078	0705	Male	No	Penitentiary
079	0201	Male	No	Penitentiary
088	0201	Male	No	Penitentiary
091	0201	Male	No	Penitentiary
093	0201	Male	No	Penitentiary
108	1302	Male	Yes	Asylum
148	1302	Male	Yes	Asylum
157	1302	Male	Yes	Asylum
167	1302	Male	Yes	Asylum
176	1407	Male	Yes	Asylum
177	1407	Male	Yes	Asylum
179	1407	Male	Yes	Asylum
124	1001	Female	Yes	Asylum
126	1002	Female	No	Asylum
145	1001	Female	Yes	Asylum
151	1109	Female	Yes	Asylum
167	0904	Female	Yes	Asylum
168	0903	Female	Yes	Asylum
172	1001	Female	Yes	Asylum
173	1002	Female	Yes	Asylum
176	1107	Female	Yes	Asylum
211	1502	Female	No	Asylum

When time relationships are considered in relation to the local secondary-like reaction it is found that regardless of size or type of inoculum the mean incubation period following intracutaneous inoculation is 9.8 days; while the mean of the 32 developing the "local secondary-like reaction" is 8 days. Of the group of 10 inoculated directly into the scar of the previous infection each patient developed clinical evidence of reinfection at the site of inoculation within the first 7 days after inoculation. Upon review of all the groups of patients inoculated intracutaneously with respect to the clinical behaviour of an entire group inoculated on a given day, it was our impression that it was uncommon in the 17 groups so injected for the first time, to see the uniformity with which all patients in a single group of this size inoculated at one time developed the lesion of reinfection in such a uniform, rapid fashion. While it may be difficult to document, on account of lapses in time of observation and the necessity for interpolation, review of other groups challenged by reinoculation at different sites gave the impression of a similar very rapid and relatively uniform development of lesion in the larger part of a group so inoculated on any given day.

If the allergic state is operative in reinfection, it is then entirely probable that allergy of the skin is responsible for a very rapid clinical response at the site of inoculation upon reinfection and can also explain the clinical picture which we called the "local secondary-like reaction".

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The statement is found repeatedly in the literature that the lesions of reinfection take the shape, in general, of the stage of infection of the experimental subject at the time of treatment of the disease (66). While our experience is not large enough to give clear cut answers we do have some opinions. The lesions of reinfection in patients treated in the primary or secondary stage appeared essentially similar to those following first infection with the notable exception of development of the local secondary-like reaction. If this latter type be considered as truly secondary, then there would appear to be a possible confirmation of the general statement, at least in that an accelerated reaction was noted which, in many cases, was highly similar to the generalized secondary. It is our opinion that in this case our findings are in accordance with the hypotheses propounded earlier in the literature.

It is furthermore worthy of noting that the single biopsy specimen taken from the "local secondary-like reaction" was read by the pathologist as "showing miliary gummata in the skin". The pathologist knew nothing of the history or clinical findings in the patient from which the specimen was taken.

A number of patients were challenged, both with and without prior treatment by us with adequate dosage of penicillin, whose diagnosis, without respect to treatment status, was latent syphilis. According to our interpretation these individuals had in the course of their infection gone into the latent stage. Some were those whom we had inoculated and who had infection of less than 1 years duration. The median time of treatment

of all experimental patient groups was 120 days. It can be assumed that those with naturally acquired latent infections were of longer duration than any of those produced experimentally. In this group of patients, some of whom showed lesions of a local secondary-like type, a few showed deep, punched-out, clean lesions with rather extensive tissue destruction which clinically resembled gummata in their evolution and in response to specific penicillin therapy. Others, though, showed only a papule highly similar to that observed in some following initial inoculation, and others of this group developed, after papule formation, generalized secondary syphilis. So while in this case the rule would not appear absolute, the clinical findings would suggest a tendency for reinfection, in the patient whose syphilis had gone into the stage of latency, to assume a gummatous or a secondary-like form as well as a pure primary form. Here, too, there appears evidence of tissue allergy which is related to immunity and a wide range of clinical variation.

It is perhaps pertinent to state that in doing darkfield examinations on our patients we were usually able to establish a darkfield diagnosis with little difficulty in patients infected for the first time. In those reinfected, it was our experience that we seldom found fields teeming with organisms, and frequently had to content ourselves with the finding of but one or two organisms after prolonged search. In this group, it often happened that in spite of search of 30-60 minutes each on samples taken from a given lesion, and on repeated attempts, we would be unable to find any organisms. In these circumstances, though, the clinical and serologic progressions established beyond doubt the presence of infection.

If absence of organisms in sufficient quantity to permit darkfield identification can be construed as evidence of tissue immunity then the statement can be made that in these reinfected individuals both clinical manifestations and darkfield findings suggest alterations in the allergic (immune) state of the skin as the result of previous infection even though the patient can be reinfected with homologous or heterologous strain. The gradual disappearance of T. pallidum from the chancre during the normal course of evolution has been considered, in the sexually acquired infection, to be a response to development of immunity within the body, and would appear to represent the developmental stage of the phenomenon which we observed upon challenge of previously infected individuals in whom the immune or allergic status has been established by the first infection.

SUPERINFECTION

Superinfection is defined as the ability to establish a new infection in animal or man still presenting clinical or serologic signs of syphilis and still retaining T. pallidum in the body. Reinfection is the development of infection in the subject biologically cured of the original infection. The problem has been discussed and summarized at length by Chesney, Urbach, and Moorman (2, 50, 66). A great deal of experimental and clinical evidence has been brought to bear on the subject, and much confusion still exists. The question has importance in the matter of cure, for it has been held by some that the ability to produce a lesion following exposure of the cured subject to infective material constitutes proof of cure of the original infection. If immunity to reinfection depends upon presence of the organism in the body, and if immunity does not persist after bacteriologic cure, then ability to reinfect constitutes

proof of cure of the first infection. But, under these circumstances, inability to produce evidence of infection, particularly in the individual treated for late syphilis, would then be construed as inability to cure late stages.

However, if immunity can be said to result from the presence of infection, then inability to produce infection after challenge of an individual treated for late syphilis can be said to present persistance of immunity without persistance of infection. The question of immunity has been examined epidemiologically by Arnold et al. (67) There it is shown that reinfection is less common in individuals treated for secondary than primary syphilis, while relapse is more common in that group. For the primary syphilitic the opposite is true. This finding is explained by the development of immunity with aging of the infection. It has been generally agreed that reinfection cannot be accepted as unequivocal evidence of cure of the first infection, but unfortunately the data reviewed in the literature are often conflicting.

We did not attempt to repeat the studies on superinfection which are reported in the literature, for it seems very well established that when the individual is challenged by heterologous or homologous strain following the second week of the chancre the incidence of lesions interpreted as superinfection is small. There seemed no need to repeat the previous work. We did, however, want to investigate the behaviour following inadequate penicillin therapy. A total of 26 patients were given what

was considered essentially inadequate penicillin therapy, 25,000 units of aqueous penicillin every 2 hours for 6 doses, total of 150,000 units, or a single injection of 300,000 or 600,000 units of POB or procaine penicillin in oil. Under these circumstances the patient could be expected to sustain a blood level of therapeutic nature for 12-24 hours. In view of the fact that the original attempt to bring about relapses with a single injection of 300,000 units of POB produced a number of cures, a group was prepared with the total dosage of 150,000 units of aqueous penicillin outlined above, and 8 patients were so treated at one time, Table 30. At 18 days after treatment, all were reinoculated with an injection of 1.04×10^6 organisms given intracutaneously into the deltoid region of both right and left arms. An equal amount of the heat-inactivated preparation was given into the forearm. The strain used was homologous, the Frew, and the patients had all been given treatment 18 days before challenge, so that there was the certainty that no penicillin remained in the body. Seven of the 8 had been inoculated 45 days before treatment, and the 8th, 125 days before, and all had had unequivocal clinical serologic, and in some cases darkfield evidence of the first infection. Upon challenge, all patients showed papules at each injection site which gradually faded, in 6, between the 2nd and 5th day, in the 7th, between the 11 and 19th day. All of this group of 7 patients showed evidence of brightening of the scar of the initial infection from 19 to 25 days after the challenge, or development of secondary lesions, and associated with this clinical observation there was found a rapid rise in serologic titer either preceeding or following the clinical manifestations.

TABLE 30

Superinfection Experiment

Patient Number and Sex	Strain		Inoculum Size		Method of Inoc.		Duration of 1st Infection before Treatment	Time from Treatment to Challenge	Clinical Reaction	Time to clinical reaction after challenge	Time to sero-reaction
	Original	Challenge	Original	Challenge	Original	Challenge					
142F	Frew	Frew	10^6	10^6	Intracut.	Intracut.	125	18	Brightening of scar and growth in size.	15	27
176F	Frew	Frew	10^6	10^6	Intracut.	Intracut.	45	18	Local secondary - like reaction with lightening around scar.	18	16
177F	Frew	Frew	10^6	10^6	Intracut.	Intracut.	45	13	Brightening of scar and growth in size.	13	5
180F	Frew	Frew	10^5	10^6	Intracut.	Intracut.	45	13	Scar pigmented and indurated	15	16
186F	Frew	Frew	10^5	10^6	Intracut.	Intracut	45	18	None	—	6
139F	Frew	Frew	10^5	10^6	Intracut.	Intracut	45	18	Mucous patches	22	16
197F	Frew	Frew	10^5	10^6	Intraven.	Intracut.	45	18	Mucous patches	13	27
200F	Frew	Frew	10^5	10^6	Intraven.	Intracut.	45	18	Reinfection or superinfection	Disappearance of lesions 11 days after challenge then reappearance after 25 days. STS inconclusive but did not show clear fall to negativity as successful treatment would be expected to give with early syphilis	

Since in some of these patients there was never complete return to seronegativity in the interim between inadequate treatment and the sequence of events following attempts to superinfect; it was necessary to arrive at a means of determination of the development of sero-positivity following challenge. Each record was inspected; and the date of evidence of sero-reactivity following challenge was obtained by interpolation between the time when the serologic titre had reached the lowest stage after treatment and the time thereafter when a sustained rise in titre of one or more dilution tubes was noted.

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Comparison of the time-interval between challenge and serologic rise reveals an incubation period averaging 16.1 days compared with that of 22.9 days for patients unequivocally reinfected following adequate penicillin therapy. The clinical manifestations subsequent to the challenge, were different from those observed following reinoculation of those patients given adequate penicillin therapy. The incubation period of those challenged with 10^6 organisms after adequate therapy was 5.9 days. But this group being subjected to an attempt at superinfection had an incubation period of 17.7 days to clinical manifestations of a sustained nature. The clinical findings were further found to occur at the site of the first inoculation or systemically rather than at the site of challenge and appeared to us to represent lesions of clinical relapse. As these patients are reviewed in light of the behaviour of the entire group studied, particularly those treated so as to produce relapse, it is our opinion, as a result of the clinical nature of the lesions, the differences in time-relationships between clinical and serologic response between this group and those reinfected following adequate therapy and those relapsing after inadequate therapy, that the findings in this group are compatible with relapse. The clinical and serologic findings in the 7 are those of relapse while the gradual fading of the lesions observed at the site of challenge represents failure of superinfection to follow challenge injection. Thus we interpret our experience to mean that superinfection in the inadequately treated patient when challenged with a homologous strain did not take place. These findings are compatible with those reported in the literature in which superinfection of the untreated early syphilitic is uncommon except after massive doses and preferably with a heterologous strain.

There was one patient of this group, No. 200F whose outcome is not certain. She showed disappearance of the lesions at the challenge sites by the 11th day, but on the 25th day after challenge there was reappearance and persistance of the papules at the site of inoculation. Darkfield examination was never positive, and the patient was discharged on the 149th day, before the outcome could be completely observed. The papules persisted through the 94th day, but there was no rise in serologic titer which is expected to occur in either relapse or reinfection. However, the blood did not become negative as would be expected if treatment were successful, and the low, persistant titer observed was of the type which has been found by us to signify possible failure in the period of 120-150 days after treatment. The clinical response at the site was longer than that we ever observed after failure of infection; the disappearance then reappearance of lesions followed the pattern of successful inoculation; so that in spite of the atypical, indecisive serologic response it must be concluded that the patient was infected.

Fleming and Waring (69) have demonstrated that in apparently immune animals challenge will result in the formation of a local papule which will spontaneously regress. Similar experience has been reported by Reynolds (70) and Turner (71). It appears from Reynolds' work that the T. pallidum is confined to the site of inoculation, does not spread to the regional nodes, and multiplies slowly if at all. Apparently in time the organisms then die out.

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This has been interpreted as a reflection of immunity. The local or systemic immunity is not sufficient to destroy the organisms at once but is sufficient to prevent dissemination away from site of inoculation and, apparently, either to prevent multiplication or else to hold it to a low level until the organisms either die or are destroyed with increase in immunity resulting from bodily response to the antigenic stimulus.

It may well be that patient No. 200F illustrates the operation of such local immunity in which there was only a local reaction. The lack of serologic response can be interpreted as further proof of lack of generalization of T. pallidum which would have resulted in the characteristic serologic rise of infection or reinfection. It is unfortunate that it was impossible to continue observation beyond 149 days so as to secure a clear-cut result. However, three such individuals of the Sing Sing group in which complete observation was possible suggest that the hypothesis is probably a valid one. (54)

Whether she represents a case of superinfection or reinfection cannot be certainly stated. For from reports in the literature (52) as well as from our own experience (table 4) it is known that primary syphilis will in about 20% of cases be cured by doses of 300,000 units of penicillin or less. If it were assumed that she had been cured, then the lesion could be called reinfection. But if it is assumed that she was not cured, then she must be considered as a case of superinfection. As she represents the only individual showing this response, and as observation on her was not complete it is impossible to evaluate the significance of her clinical and serologic course.

It would appear, though, from our findings, that superinfection by homologous strain in early syphilis of more than 63 days duration is at least an uncommon event.

Latent Syphilis

The diagnosis of latent syphilis is one which is surrounded by many difficulties. In fact, in some ways it is a negative diagnosis and also one which must be made with much caution and with thorough knowledge of the patient so as to relate the serologic findings with both history, physical findings, and other laboratory data. Simply stated, latency is the stage of syphilis which supervenes after the disappearance of all early lesions of the disease, the relapsing secondary, the secondary, or the primary, and is characterized by the following:

1. Positive STS.
2. Negative spinal fluid findings.
3. Absence of signs or symptoms of early or late syphilis.

In light of research work done since this study was completed it may be added that the state is accompanied by the presence of a positive TPI test (68).

Strictly speaking, the diagnosis is made without reference to the treatment status, so that by some definitions, the patient successfully treated for syphilis in any stage, early or late, who has shown disappearance of all signs and symptoms of syphilis can be diagnosed as having latent syphilis so long as the STS remains positive. But from the point of view of clarifying the discussion of the significance of the positive STS it is probably helpful and preferable to discuss the serologic status of the patient with respect to what is known about the treatment status. The appellation of latent syphilis will be reserved

for this discussion to the condition found in patients with a positive STS by both the VDRL slide and Kolmer standard tests, with no signs or symptoms of syphilis, and either untreated or inadequately treated.

As will be discussed later in this section, the serologic diagnosis of latency was made if the patient had positive VDRL slide and Kolmer standard tests, regardless of the results of the Kahn and Massini. (But in this group, in contrast to those treated successfully for early syphilis and returning to the seronegative state, the occurrence of a positive VDRL slide and Kolmer standard in the presence of negative Massini and Kahn was not observed.) The finding of a positive or doubtful Kahn or Massini in the untreated patient with negative VDRL and Kolmer was considered as representing false positivity.

According to the commonly accepted theories of the course of untreated syphilis, as recently documented by the Brumsgaard and Tuskegee studies (58, 59), syphilis will show one of the following courses:

1. Return over the period of years to seronegativity, apparently representing spontaneous cure, without development of clinical evidence of late manifestations.

2. Remain seropositive until death, but reveal at death no evidence of late manifestations. (This apparently represents bacteriologic cure which is followed by the serofast state.)

3. Develop late manifestations of syphilis, of benign or serious type, such as gummatous, cardiovascular, or CNS syphilis.

In the latter state of the third category, it is sometimes found that the patient with late lesions of syphilis will show seronegativity by one or more of the common serologic tests (but probably not by the TPI test). Clinically it is rare to see negative STS of the blood in the face of an active spinal fluid (Dattner-Thomas type) (60). The negative STS is more commonly found in cardiovascular syphilis or tabes in which the late, symptomatic lesions may represent the end products of scarring—tissue damage and healing—which result even though the body may have eliminated the T. pallidum by spontaneous bacteriologic cure.

It has been our experience that reinfection of a patient cured of primary or secondary syphilis by adequate doses of penicillin can be experimentally carried out within the limit of 1 week to 1 year between treatment and challenge. This is possible regardless of the duration of experimental primary or secondary syphilis, whether the challenging dose was a homologous or heterologous strain, and regardless of the interval between administration of penicillin and challenge, so long as the body was penicillin-free at the time of reinoculation. It may be added that certain of the patients challenged after experimentally induced infection were those whose infection had been latent, latent in the sense that there were no physical signs of symptoms of syphilis, or none other than lymphadenopathy found to accompany the development of the seropositive state following inoculation which was interpreted to mean infection. These patients with latent syphilis, experimentally inoculated, can be considered as representing the stage of early latency, i.e., less than 4 years duration.

In patients experimentally inoculated and given subcurative therapy, superinfection, with homologous or heterologous strain was perhaps not possible and at least highly uncommon when given after the patient had had his infection of 63 or more days. In this finding we confirmed the classical findings that superinfection could not commonly be accomplished in man after the chancre of infection is well developed. (2) In experimental animals, superinfection is reportedly possible if the challenging dose is massive or if it consists of a heterologous strain. (50, 66) The ability to bring about reinfection or superinfection is dependent, then, upon the age of initial infection, the strain, and the site of reinoculation. It is rather generally agreed that superinfection in the latent stages in man is difficult or rare, although it is stated that in the tertiary stages of disease superinfection or reinfection is perhaps more common possibly indicating, a lowering of immunity. (50,66)

In fact spontaneous cure of the individual which takes place in about one third of untreated persons suggests that a high degree of resistance should remain after this cure. However, the individual who progresses to the development of the late stages can be assumed to lack the ability to muster sufficient immune forces to cure the infection, so that there occurs at intervals a release of the organism from the restraining effect of the immune mechanism with the production of signs and symptoms of disease. This suggests possible defects in the immune mechanism of the individual and suggests why it has been found possible to produce lesions suggestive of superinfection or reinfection in paretics and tabetics.

It is our experimental finding, in the patient inoculated for the first time or reinoculated after successful treatment for early syphilis, that the appearance of a lesion at the site of inoculation indicates with a high degree of certainty that the patient has been infected. This relationship between inoculation and lesion, while accompanied by, and apparently causing development of or increase in titre of seropositivity, can be considered, in our experience, as diagnostic of infection without reference either to the STS or the demonstration of T. pallidum by darkfield examination. This concept is of extreme importance in consideration of the findings which follow.

As mentioned earlier, as soon as we began to do large-scale serologic testing upon unselected population groups we were struck by the finding of a high degree of seroreactivity by the STS using lipoidal antigens in flocculation or precipitation tests. This contrasted with lack of reactivity in the same sera when tested by the complement fixation test using lipoidal antigen or the VDRL slide test using a cardiolipin-lecithin antigen. Discussion of these findings is carried out in other papers (9-16). It was our hypothesis after clinical study that it was highly probable that this serologic discrepancy, in those patients without history of treatment of syphilis represented false-positivity.

For without a history of recent treatment for early syphilis, in which the discrepant results would be expected (61) it was certain that these patients were not showing serologic evidence of treated early syphilis.

By the same reasoning it seemed that they did not represent treated or untreated latent syphilis. For the pattern of serologic findings was not that observed in adequately treated or spontaneously cured latent syphilities (61,64).

In order to test this hypothesis groups of patients with negative VDRL and Kolmer and positive Kahn and Mazzini tests and with no history of syphilis, considered as non-syphilitic, and groups in which all tests were positive, with or without history of syphilis or treatment but without clinical evidence at the time, and considered latent, were challenged. All of the first type developed syphilis, while none of the latter type developed clinical evidence. Thus early in the course of studies it was determined what was the possible significance of these discrepant findings. Throughout subsequent experimental studies numbers of patients having the second type of serologic finding, not related to history of disease or treatment, and considered as latent syphilities, were inoculated. It was found that these patients with negative VDRL test and positive or doubtful Kahn or Mazzini, showed no immunity to inoculation, for infection took place at the same rate as in those patients completely seronegative at the time of first exposure to infection.

In contrast, the individuals considered as latent syphilitics as a result of positive VDRL slide and Kolmer tests showed some resistance to infection as catalogued in Table 32. This resistance was considered by us to indicate that the individuals we considered as latent were just that.

With these findings, then, it became possible to draw up the following hypothesis: In this group of people, the occurrence of a positive or doubtful Kahn or Mazzini test in the face of negativity of VDRL slide and Kolmer, and in absence of history of treatment for syphilis, meant that the tests represented false positive STS. For, on the basis of our experimental work it was considered that ability to produce infection in such patients meant that the patients had no immunity to syphilis and thus did not have syphilis at the time of inoculation. (we have reason to think that superinfection in man is so uncommon as not to require consideration.) For in the group with this discrepant serologic finding, observed for periods of months, without either treatment or challenge, we did not see the type of serologic progression which would have occurred if the initial findings had represented early syphilis in the pre-lesion period, and which would have been characterized by a steady increase in seropositivity. (72) Nor did we see in similar groups evidence of, or development of lesions of late syphilis.

It was next necessary to study the significance of the positive VDRL slide and Kolmer tests. Following experimental inoculation accompanied by the development of darkfield positive primary and secondary lesions there was an invariable development of seropositivity by all tests of the battery used, including all mentioned in this discussion. Our experimental findings were in complete harmony with the clinical acceptance of the relationship between syphilis and seropositivity in early syphilis. But in surveys of the groups with which we worked we found numerous individuals

positive by all tests used, some with a history of lesions of syphilis which healed spontaneously, some with no history of any lesions, some with history of treatment with arsenic, almost always inadequate (less than 20 injections of arsenic and 20 of bismuth), and a few with clinically proven neurosyphilis or congenital syphilis. As is the finding in the United States, and in most of the world, as well as in this group, it is unfortunate that the majority of patients with seropositivity have no history of infection, so that the clinician has difficulty in deciding the significance of the findings. Again, at the time this work was done, the TPI test was not yet developed, so that our study was carried on in an attempt to throw light upon the significance of the positive VDRL slide and Kolmer tests. For as stated in the preceeding paragraphs, we had decided that in the face of lack of history of treatment and clinical evidence of syphilis, the positive or doubtful Kahn and Mazzini tests alone probably signified false positivity.

In view of our experimental findings we believed that the ability to produce a lesion following inoculation signified production of infection, and thus freedom of the body from T. pallidum prior to inoculation. In studying this problem in the patient with late latent or late syphilis we were obliged to make the diagnosis upon serologic grounds with but few exceptions which will be mentioned later.

In view of our findings with regard to the serologic discrepancy in the absence of history of treatment, we came to the conclusion that the patient with positive VDRL slide and Kolmer test, whether or not accompanied by reactivity in any other tests of the battery used, had or had had latent or late syphilis. (Treatment may have been given, in which case the positivity represented evidence of the disease treated at the aforementioned stage.) It must be remembered that in the group with which we were working, none of the other treponemal diseases complicated the picture, for Yaws has not existed in the country for several decades, and none of the patients in the general population of the institutions revealed evidence of scars or active late lesions of yaws. Pinta was not involved for to our knowledge, none of our patients came from that very small area of the country to which it is confined and in the institutions we saw no patients with cutaneous lesions of any stage of pinta, even though we searched for and were completely familiar with the clinical manifestations of the disease.

Notes on accompanying Table:

1. The two patients showing no responses after adequate treatment did show a non-virulent transient papule or macule of approximately one weeks duration.

2. All patients in the tabulation are males inoculated intracutaneously and have not been submitted to any prophylactic measures.

3. Diagnosis of latency based upon seropositivity by each of the following tests:

VDRL slide
Kolmer
Kahn
Mazzini

Regardless of history or evidence of penile scar.

In Table 32 the results of challenge in patients seropositive to the battery of tests used is shown, with results broken with respect to the Kahn titer.

It was regrettably impossible to carry out regular, serologic observations on most of these patients, and in some, none after inoculation, but all who are listed were observed for at least 4 months to determine any clinical evidence of infection, either at the site of inoculation or systemically. In view of our findings with respect to relation between inoculation, development of lesion, and serologic evidence of infection, it can be said that absence of lesion signifies with a high degree of certainty the absence of infection. This statement has subsequently been confirmed with respect to the group of patients initially treated for latent syphilis or late symptomatic syphilis in the Sing Sing study (54).

Adequacy of treatment was based upon convention of 20 injections each of arsenic and bismuth or upon administration of at least 4,250,000 units of penicillin by the authors themselves in accordance with one of the schedules discussed under the section on treatment.

It will be seen in Table 32 that of those with 32 or less Kahn units the 6 who had had adequate therapy all revealed evidence of reinfection upon challenge. Of those having had inadequate treatment, or none at all, 12 of 18 developed evidence of infection. Of those with 64 or more Kahn units, regardless of treatment status 14 of 15 patients revealed no evidence of infection; while only 1, and that was one of the three given adequate therapy with penicillin prior to challenge, developed an infection.

TABLE 32

Responses to Virulent Inoculation of Patients with Latent Syphilis of More Than 1 Years Duration by Preinoculation Titre and Adequacy of Treatment Prior to this Study

	Adequate Treatment						Inadequate Treatment					
	32 or less Kahn Units		64 or more Kahn Units		Total		32 or less Kahn Units		64 or more Kahn Units		Total	
Response	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Unresponsive	0	0.0	2	66.7	2	22.2	6	33.3	12	100.0	18	60.0
Responsive	6	100.0	1	33.3	7	77.8	12	66.7	0	0.0	12	40.0

* In this instance any clinical or serologic changes that provides definite evidences that a reinfection has occurred.

Although the diagnosis of latent syphilis had to be made upon serologic findings alone, without recourse to spinal puncture in this group, and without the availability of the TPI test (68) which would have established a relatively unequivocal diagnosis of latency; we feel that the diagnosis can be considered as probably correct with a high degree of certainty. This we say because of the following facts:

1. In groups of patients of same age and nationality and general background where spinal punctures could be done, it was uncommon to find positive spinal fluids.

2. Our studies in early syphilis and our epidemiologic work throughout the country gave rather clear cut evidence of the high degree of specificity of the VDRL slide and Kahn tests.

3. Subsequent studies in the United States; and through the WHO have confirmed the high degree of specificity of the VDRL slide test.

We have further reason to believe that in this group of patients the disease was probably of more than 1 years duration.

Accepting them, the hypothesis that the group shown in Table 32 represents latent syphilis, of more than 1 years duration, we can come to several conclusions:

1. In an individual with latent syphilis of more than 1 years duration, with or without treatment, a Kahn titer of 32 units or less indicates that there is a relatively low degree of immunity to reinfection. While the patient may sometimes be resistant to challenge, if he has had inadequate or no therapy, it would seem that under this circumstance,

there may be either persistance of infection with associated resistance to superinfection, or some degree of immunity following an infection which has undergone spontaneous cure. But it would appear that if adequate therapy has been given the individual is highly susceptible to reinfection. Thus, in the untreated or inadequately treated individual, a finding of this titer suggests that the patient may have possibly undergone spontaneous cure, or else have a low degree of resistance to superinfection.

2. In a latent syphilitic with or without treatment, the finding of a Kahn titer of 64 units or more indicates that the patient will be highly resistant to a challenge; whether that challenge represents potential reinfection or superinfection. While the numbers are small; the fact that the only success in that category was found in a single patient having had adequate penicillin therapy suggests, if we assume that superinfection is not common, as shown elsewhere in this paper, that a titer of this magnitude signifies either active, latent infection; or a relatively high degree of immunity following adequate therapy.

In view of the facts that reinfection could be accomplished following therapy for primary and secondary syphilis regardless of the titer; and that ability to produce reinfection in the latent syphilitic is apparently related to the serologic titer and treatment status; it would seem that when the stage and treatment status of disease are considered the titer is of value as an index to either activity of the infection or to immunity. The titer of 64 or more units, persisting, after adequate therapy for latent syphilis indicates probable refractoriness to reinfection with

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heterologous strain and thus immunity. A titer of 32 Kahn units or less under the same circumstances is an index of only partial immunity. When we consider that in this group some had had previous small doses of arsenic or penicillin for therapy, inadequate, by usual standards, but in some cases probably curative (39,63) the susceptibility of these patients may in part be explained. Furthermore, spontaneous cure of syphilis can take place, in about a third of those infected. Cure, whether spontaneous or induced by effective therapy in the patient with syphilis beyond the early latent stage is accompanied by a gradual decline in seropositivity in the group and often in titer of the individual patient. (62,64) Finally, in general, in any infectious disease in which there is not repeated exposure to the infective agent, there is seen a gradual decrease in resistance. In diseases such as scarlet fever or diphtheria epidemiologic evidence shows a highly significant relationship between the decline in antibody content of the individual blood and susceptibility of the group being studied of which he is a part to reinfection with passage of time (65). In the syphilitic man there is some evidence from clinical experience to suggest that with passage of time after successful treatment of the later manifestations of syphilis there may be gradual loss of immunity (74).

Thus, our findings would suggest that the lower titer in the group showing susceptibility to reinfection (which we consider to have taken place, rather than superinfection) indicates a lowered degree of immunity. It is thus evident, if our hypothesis is correct and if we have interpreted our findings correctly, that the serologic titer can be considered as indicative of immunity, but only when correlated with the stage of infection with which the titer is found. For the titer of 64 in early, syphilis, of less than 2 years duration, was not found to be accompanied by resistance to infection, as it was in syphilis of more than 2 years duration. And the titer of 32 or less units was similarly found to have a different significance in the two time periods.

(Note. It is important to keep in mind that the Kahn titer here in the test performed according to reference 18 is set at the level of antigen sensitivity maintained by the VDRL of the USPHS from 194_ to present time (1954), and is the level which has been kept standard for the Blue Star Study (75) of treatment of syphilis carried out by the Division of Venereal Disease, USPHS.)

It would thus appear that reagin gives evidence of real value in discussion of the immune status of the individual when related to the duration of infection in man. Experimental animal studies have indicated, in the same way, increase in immunity with increased duration of infection. (73)

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The studies in immunity can be summarized as follows:

1. With challenge doses of the order of 10^5 organisms no immunity to reinfection was found to follow primary or secondary syphilis.

2. In latency of more than 1 years duration with or without treatment prior to challenge, a Kahn titer of 32 units or less indicates that there is a low degree of immunity to reinfection (or superinfection) as massive challenge often results in infection.

3. In the class of 2 above, but with a titer of 64 Kahn units or more, reinfection upon challenge is uncommon. In these circumstances, the Kahn titer appears, when considered with clinical background, to be an index of immunity.

4. Following treatment of primary, secondary or early latent syphilis of less than 1 years duration, reinfection is possible by homologous or heterologous strain regardless of Kahn titer.

5. In reinfection there is noted more frequently than in first infection a phenomenon called "local secondary-like reaction". The lesion of inoculation is surrounded by a sharply localized maculo-papular eruption clinically identical with the eruption of generalized secondary syphilis.

This is considered to represent a manifestation of allergy resulting from the course of the first infection.

6. In some lesions of reinfection in latent syphilis, treated or untreated, the lesion of inoculation was clinically a gumma. This is also interpreted as a manifestation of allergy or incomplete immunity.

7. Symptomatic infection was more common in reinfection than after challenge of the first infection. This suggests again that the skin becomes sensitized by infection.

8. Adequate controls were provided to assure that the sequence of events described represented infection and not simple reaction to foreign protein.

9. There was evidence to suggest more rapid development of lesion in reinfection than in first infection when comparable methods and doses were used.

10. Skin and mucous membrane, when intact, present a barrier to infection with T. pallidum.

11. Individuals exposed for the first time to T. pallidum introduced into or under the skin have little or no resistance to infection if sufficiently large inoculum is used.

12. Infection can be obtained by injection of as few as 10 T. pallidum, and above an inoculum of the order of 10^4 by injection susceptibility upon first exposure is almost absolute.

13. Sufficient patients not infected by one technic were reinfected by same or different methods to suggest that whatever immunity may be shown to first challenge is relative rather than absolute.

14. The finding after treatment of lack of immunity to large challenge dose more pronounced in early than late syphilis suggests that primary or secondary infection does not confer a high degree of immunity to massive challenge.

On the other hand, clinical observation suggests that immunity to normal risk of infection does follow and is greater as direction of disease increases.

15. The "heroic" challenge methods used tend to overcome low grades of immunity and are not an accurate index of what may be expected in normal sexual experience. However, we were interested in establishing the fact of absence or presence of immunity. We felt that our experiences indicated that the details could better be worked out in rabbit studies.

16. Our experience leads to the conclusion that superinfection is an uncommon occurrence, if it happens at all. Our findings could be interpreted as only showing it to be at most, a rare occurrence, but could not be interpreted as proving or disproving it.

17. Incubation period to development of lesions was usually slower in penile mucosa than in skin when identical means of inoculation was practised simultaneously in the two sites in the same individual. This is considered as possible evidence of either difference in resistance to infection or in reactive potential in the two tissues.

18. The intact mucous membrane barrier was not affected adversely by use of mucin in an attempt to increase rate of infection.

19. There was no difference in immunity to infection which could be related to strain or source of T. pallidum, whether human or rabbit.

Summary

Following penile inoculation, classical hard chancres as well as all other types of penile lesions observed in sexually-acquired syphilis were found.

The type of local lesion occurring after inoculation into the skin was characteristically a nodule or papule which might occasionally ulcerate.

Secondary syphilis of the types seen in clinical practice was frequently observed.

There was no clinical or serologic difference observed which could be related to any strain of T. pallidum either animal passed or human passed.

Asymptomatic infection could be produced by intravenous inoculation. It was found to occur following any type of inoculation, was more common after failure of local prophylaxis. It was more frequently observed after inoculation through mucous membrane than through skin.

Asymptomatic infection did not seem to be produced with abnormal frequency as compared to controls by failure of systemic prophylaxis with penicillin, arsenic, or bismuth.

Lymph node enlargement was found to accompany about half of the cases of primary and secondary syphilis. It bore no relationship to strain, type of inoculation or sex.

Completely asymptomatic infection with no observable lymph node enlargement was repeatedly observed.

There is no sexual difference in rate of asymptomatic infection following intracutaneous inoculation.

Failure of locally applied prophylactic agents predispose to development of asymptomatic infection.

Failure of systemic prophylactic agents, penicillin in our studies was not marked by a higher rate of asymptomatic infection than observed in control inoculation.

The type of clinical response is, in part, conditioned by the tissue into which the organism is introduced.

Wide individual differences are found in comparing lesions in different patients given identical inocula.

There is no evidence of strain differences or differences referable to animal passage in respect to clinical lesions or serologic response.

The incubation period following use of a given inoculum varies inversely with the size of inoculum and shows variations depending upon method and site of inoculation and upon the individual.

Reinoculation could be practiced successfully at different sites or even into the scar of the original lesion.

In general from clinical appearance alone it could not be stated whether or not the lesion represented reinfection except in the face of the "local secondary-like reaction".

However, a lesion designated by the term, "local secondary-like reaction" was found significantly in reinfection, practiced by intracutaneous injection into arm or forearm. In this, the lesion of inoculation was surrounded by a border of macules or papules, or both, identical with those of secondary syphilis. In some instances, a generalized, classical eruption supervened. In others the lesion remained localized.

In a few cases of reinfection in patients who had passed into the latent stage the lesion of reinfection resembled a cutaneous, solitary gumma.

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