

STUDIES IN EXPERIMENTAL SYPHILIS.

II. THE INFLUENCE OF A NON-SPECIFIC INFLAMMATORY REACTION UPON THE DEVELOPMENT OF THE CHANCRE.

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PLATES 19 AND 20.

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INTRODUCTION.

In considering the factors that govern the initiation of syphilitic infection, whether in man or in animals, most of the emphasis has in the past been placed upon the occurrence or non-occurrence of mechanical defects of the skin or mucous membranes. There has been considerable discussion as to the necessity of a break in the continuity of the surface epithelium of these structures to permit *Treponema pallidum* to gain entrance to the body of the host. Experimental work in this field has demonstrated conclusively that in the case of the rabbit virulent strains of *Treponema pallidum* can produce a characteristic lesion on a mucous membrane surface which is intact so far as can be seen from macroscopic examination.^{1,2} Furthermore, such invasion may take place without the development of a characteristic local lesion at the site of inoculation.²

Other factors which might conceivably influence the initiation of syphilitic infection have received attention, as, for example, the comparative availability of various sites or tissues. In this respect it has been shown that some tissues (testicle, cornea) are more favorable for inoculation than others (subcutaneous tissue), and that in a given tissue the mode of inoculation may, under conditions not

¹ Reasoner, M. A., *Am. J. Syph.*, 1917, i, 478.

² Brown, W. H., and Pearce, L., *J. Exp. Med.*, 1924, xxxix, 645.

perhaps clearly understood as yet, influence appreciably the course of the primary syphilitic manifestation. Thus, in a given series of rabbits inoculated intracutaneously with equal amounts of the same virus, some animals will develop a characteristic lesion at the site of inoculation and the organisms will be disseminated throughout the body, whereas other animals will develop only slight lesions or even none at all, and still infection and dissemination will have taken place.³ It is clear, therefore, that in speaking of the initiation of syphilitic infection the distinction must be made between the occurrence or non-occurrence of a chancre at the portal of entry and the occurrence of infection, since the two have not been proved to be mutually dependent.

It seemed to us that if it were possible to obtain any knowledge of the factors which operate to suppress the occurrence of a lesion at the portal of entry, a better understanding might be had of the mechanism of resistance to syphilitic infection in general. It seemed worth while to approach this problem by seeking to determine the influence of a non-specific inflammatory reaction upon the initiation of syphilitic disease. This particular question has not, so far as we have been able to ascertain, received any attention from workers in this field.

There is some evidence to show that under certain conditions a non-specific inflammatory reaction involving the skin and subcutaneous tissues may constitute a focus which is highly resistant to infection with some types of bacteria, while, on the other hand, it may constitute a focus which is distinctly favorable to infection with other types of disease-producing agents. Some unpublished studies by one of us (Chesney) on the bacterial flora of healing wounds in human beings which had not been treated with Dakin's solution and healing experimental wounds in rabbits suggested that a healing wound is not a particularly favorable site for the growth of the ordinary microorganisms contaminating the skin. On the other hand, the work of Teague and Goodpasture,⁴ who succeeded in producing typical lesions of herpes zoster upon the skin of guinea pigs which was already the seat of an acute dermatitis produced by coal tar, indicates that skin which is the seat of an inflammatory reaction may constitute a very favorable site for the establishment of infection with the virus of herpes zoster.

³ Chesney, A. M., *J. Exp. Med.*, 1923, xxxviii, 627.

⁴ Teague, O., and Goodpasture, E. W., *J. Med. Research*, 1923-24, xlv, 185.

In the absence of any information as to what might be the case in syphilitic infection, experiments were designed to test the availability of tissues already the seat of a non-specific inflammatory reaction for the initiation and development of the primary manifestation of syphilitic infection. These experiments, as will be seen later, all tended to show that an already existing non-specific inflammatory process favors rather than impedes the establishment of the experimental infection.

EXPERIMENTAL.

Experiment 1.—Two groups of adult male rabbits of various breeds were used. In the first group (Group A), comprising five animals, a circular area of skin on the back, roughly 4 cm. in diameter, was excised under ether anesthesia, exposing the subcutaneous tissue. These wounds were allowed to heal under natural conditions, no dressings or antiseptics being applied. Crusts of inspissated serum rapidly formed, beneath which granulation tissue developed. On the 11th day after operation, when the wounds were healing and the epithelium was closing in from the sides, a second group, comprising six animals (Group B), was subjected to a similar operation. 48 hours later the superficial crusts were removed from the wounds in both groups of animals, care being taken to disturb the underlying surface as little as possible. The exposed area was then inoculated with a testicular emulsion in physiological salt solution, rich in actively motile spirochetes. The Nichols strain was used in all the experiments. A drop of the emulsion was allowed to fall on the exposed surface and was distributed evenly by gently rubbing for 1 minute. Thus, the experiment offered an opportunity of comparing the behavior, toward syphilitic infection, of a granulating wound 13 days old with that of a comparatively fresh wound (48 hours old).

All the wounds healed without at first any sign of syphilitic infection. The older wounds naturally were completely healed before the more recent ones. It was noticed that there was an increased growth of hair along the edges of the wound and along the scar when the epithelium had entirely covered the granulating surface. Shortly after the wounds had healed, clinical evidence of syphilitic infection began to be manifest, at first in the old wounds, later in the recent wounds, by the appearance of induration along the line of the wound. This induration increased rapidly in extent in both planes as a rule, so that an elliptical elevated area of induration was soon formed (Fig. 1). At the same time necrosis of the epithelium in the center of the indurated area set in and increased in extent until ultimately a remarkably large chancre was formed (Figs. 2 and 3). Characteristic examples of *Treponema pallidum* could be demonstrated with ease in the serum expressed from beneath the crust of such lesions.

The incidence of positive inoculation results in the group of rabbits with old

(13 day) wounds was 100 per cent, while that in the rabbits with fresh wounds (48 hours) was 66 $\frac{2}{3}$ per cent. A distinct shortening of the incubation period was also observed in the case of the former group. These results are brought out in Table I.

Table I shows that in the group of rabbits with old wounds the incidence of positive inoculations obtained was 100 per cent and the average incubation period 25.4 days, while in the group in which a comparatively fresh wound was inoculated the incidence of positive

TABLE I.
Behavior of Recent and Older Wounds to Syphilitic Infection.

	Rabbit No.	Result.	Incubation period.	Results of node transfer.
Group A (13 day wounds).	1	Chancre.	<i>days</i> 25	Not done.
	2	"	30	Positive.
	3	"	25	Not done.
	4	"	25	Positive.
	5	"	22	Not done.
Average.....			25.4	
Group B (48 hr. wounds).	6	Chancre.	43	Not done.
	7	"	35	" "
	8	Negative.		Negative.
	9	Chancre.	33	Not done.
	10	Negative.		Negative.
	11	Chancre.	37	Positive.
Average.....			37	

inoculations was 66 per cent and the average incubation period was 37 days. In the former group the lesion on the whole attained a greater size than in the latter group, although there were exceptions to this rule.

In order to determine whether or not the two animals with recent wounds which failed to show any evidence of a syphilitic lesion at the portal of entry were in reality infected, popliteal node transfers were made to normal animals with negative results. Similar transfers from animals in each group that showed characteristic chancres were all positive, indicating that when chancres are produced on the back

in the manner described above, generalisation of the virus takes place as it does in animals inoculated on the genitalia.

The experiment seemed to indicate that a non-specific inflammatory reaction favored the initiation of syphilitic infection and, further, that a granulating wound constitutes a somewhat more favorable site for the production of a characteristic chancre than does a comparatively fresh wound. It seemed important to repeat this experiment in order to determine whether this relationship was constant, and, accordingly, a second experiment was planned which was

TABLE II.
Behavior of Recent and Older Wounds to Syphilitic Infection.

	Rabbit No.	Result.	Incubation period.
			<i>days</i>
Group A (16 day wounds).	12	Chancre.	28
	13	"	30
	14	"	28
	15	"	30
	16	"	28
	17	"	33
Average.....			29.5
Group B (48 hr. wounds).	18	Chancre.	36
	19	"	39
	20	"	44
	21	"	40
	22	Negative.	
	23	Chancre.	39
Average.....			39.6

similar in every respect to the first, except that the old wounds were 16 days old at the time inoculation was carried out instead of 13 days, as in the previous experiment. The results of this experiment were quite similar to those obtained in the first, and are shown in Table II.

Table II shows that in the group of rabbits whose wounds were 16 days old when inoculation was performed there were positive results in every instance, whereas in the group whose wounds were 48 hours old at the time they were inoculated there were positive takes in five out of six animals, or 83.3 per cent. Moreover, the appearance of the

lesions was again delayed in the group of animals with 48 hour wounds by an average of 10.1 days.

This experiment, while perhaps a little less striking in its results than the first, confirmed it and thus seemed to establish the fact that a granulating wound 13 to 16 days old is more favorable for the initiation and development of a characteristic syphilitic lesion than is a wound 48 hours old. This greater suitability of a granulating wound for the production of a syphilitic lesion is evidenced by (a) the greater number of positive takes, (b) the diminished incubation period, and, possibly, (c) the greater size of the lesion on an average.

Histological examination of the tissues involved in these 48 hour and 13 day wounds as yet uninoculated showed the following characteristics.

48 Hour Wound.—Most of the corium has been removed by the operation exposing the subcutaneous tissue. The raw surface of the wound is made up of more or less homogeneous pink-staining material in which the remains of dead nuclei are visible and comparatively few apparently living nuclei are seen. It will be remembered that prior to inoculation the crust of the wound was removed, hence, little or no fibrin is to be seen on the cut surface. Beneath this layer of necrotic tissue there is a zone composed of densely packed polymorphonuclear leucocytes for the most part, together with some large mononuclear wandering cells and lymphocytes. This layer is sharply delimited and at some points attains a thickness of almost 0.5 cm. It extends in a continuous sheet from one margin of the wound to another. Beneath this leucocyte wall the subcutaneous tissue is infiltrated with polymorphonuclear leucocytes and large mononuclear wandering cells. There are relatively few lymphocytes in this zone. The blood vessels which are most numerous at the base of the layer of subcutaneous tissue, just above the muscularis, are dilated and engorged with red cells. Beyond the wound, where the epithelium and corium are intact, the cellular reaction is not to be seen, the tissue in this area appearing normal. The epithelium at the edge of the wound is beginning to show hyperplasia.

The section shows the characteristic appearance of an early stage of inflammatory reaction in which the predominating feature is the response of the wandering cells to the stimulus produced by the injury. As yet the reparative features are in the background.

13 Day Wound.—The section of a wound such as was produced in the experiments cited above, when it has progressed 13 to 16 days toward healing is characterised chiefly by the presence of a thick layer of granulation tissue, extending

from the muscularis to the level of the intact epithelium. This layer is as much as 5 mm. in thickness in some parts. The surface of the wound is made up of a layer of fibrin containing polymorphonuclear leucocytes, large mononuclear wandering cells, and lymphocytes, although the first named predominate by far. Beneath this leucocyte-fibrin layer there are enormous numbers of fibroblasts showing a parallel arrangement for the most part, their nuclei large, pale-staining, and elongated, with prominent nucleoli. Polymorphonuclear leucocytes and lymphocytes are relatively few in this area. Many newly formed capillary loops are visible in this zone and the blood vessels at the base and margins of the granulating area are engorged. There is a striking hyperplasia of the epithelium at the margins of the wound. Here the epithelium is many times its normal thickness and its extension over the granulation tissue is beautifully illustrated.

In brief, the section of the 13 day wound shows the characteristic features of an inflammatory reaction which has progressed to the point where the processes of repair constitute the predominant feature.

It was apparent from a study of the histological picture of the two types of wounds that the cellular picture was quite different, as one might expect. In the 48 hour wounds the picture was predominantly that of reaction on the part of existing blood vessels and wandering cells, with little or no participation as yet by the reparative processes. In the 13 day wounds, on the other hand, the picture was predominantly that of activity of the reparative processes.

In view of the manifest difference in the relation of early and late inflammatory reactions to a superimposed syphilitic infection it seemed desirable to produce a series of inflammatory reactions of varying degree of severity upon the skin of rabbits in order to determine what type of reaction or what stage in the reaction is most favorable to the establishment of infection. The protocol of the experiment follows.

Experiment 3.—Six groups of adult male rabbits of various breeds were utilised. Care was taken to distribute the representatives of the various breeds, as far as possible, equally throughout the groups. For the most part the ordinary grey and brown rabbits were used.

Inoculation.—The site of inoculation was always the same—the lower dorsal and upper lumbar regions. The inoculum was in each instance the same and consisted of 0.05 cc. mixed testicular emulsion in physiological salt solution from two rabbits with actively developing orchitis. The inoculum contained numerous actively motile treponemata per field and was regarded as very rich

in these organisms. It was allowed to drop on the exposed surface and was then gently spread with the gloved finger.

Group A. Four Males. Control.—No inflammatory reaction. In this group the hair on the back was clipped but nothing was done to the skin proper. The inoculum was rubbed directly upon the macroscopically unbroken skin.

Group B. Four Males. Control Subcutaneous Inoculation.—No preparation other than clipping the hair. The bulk of the inoculum was deposited about 2 cm. from the point where the needle penetrated the skin.

Group C. Four Males. Scarification.—The animals were anesthetised with ether, the hair clipped from the back, and an area of epidermis shaved off by means of a razor, in much the same manner as when Thiersch skin grafts are cut. The exposed surface bled rather freely and some exudation of serum had occurred before the inoculations were made.

Group D. Seven Males. Preliminary Coal Tar Dermatitis.—An acute dermatitis was produced by three preliminary applications of coal tar to the hair-clipped skin of the back at 48 hour intervals. 48 hours after the last application, the animal was anesthetised and the inspissated tar was removed with forceps, exposing a raw, hyperemic surface. In peeling off the inspissated tar the epidermis was noted to be thicker than normal and some of the superficial layers of the epidermis appeared to have been removed.

Group E. Fresh Wounds. Six Males.—Under ether anesthesia an elliptical area of skin was excised, exposing the subcutaneous tissue. The excision was carried out 1 to 2 hours before the inoculation. The wounds were moistened with a few drops of saline solution just prior to inoculation.

Group F. Old Wounds. Six Males.—Under ether anesthesia an elliptical area of skin was excised 14 days prior to the inoculation. No attempt was made to keep these wounds sterile. Just before inoculation the crusts were removed with sterile forceps and swab cultures made of the granulating surface. An abscess had developed in one of the wounds, the others appeared clean. Cultures were made aerobically on rabbit blood agar plates. They were all sterile except the one from the abscess which yielded a Gram-positive coccus that grew in chains.

The rabbits were kept under observation for a period of 90 days. The results are shown in Table III.

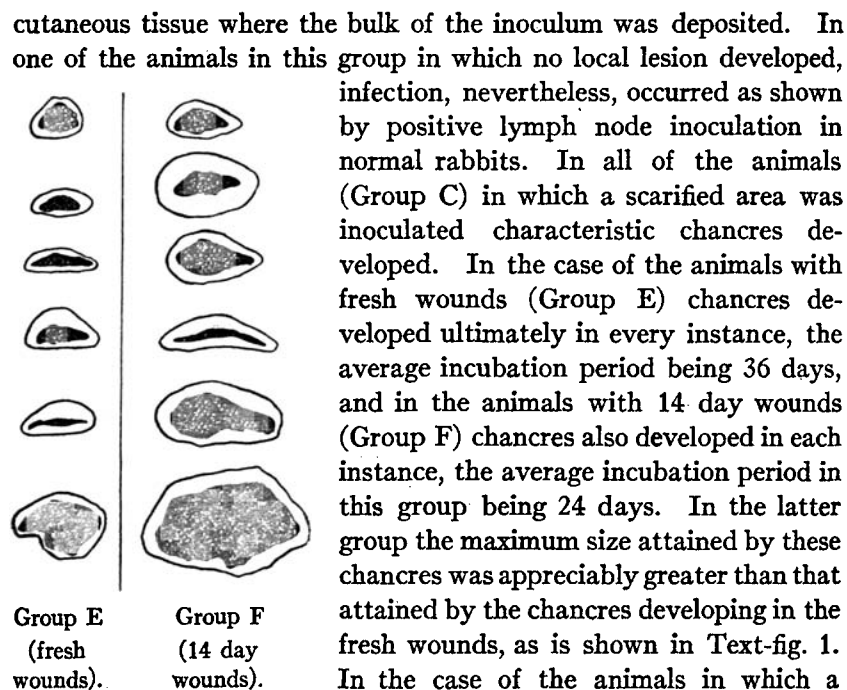
It will be seen from Table III that no animal in Group A, in which the unbroken skin was inoculated, developed lesions. In Group B, which received subcutaneous inoculations, no lesions developed in two animals, whereas in the other two characteristic syphilitic lesions made their appearance. It is of interest to note that in both of these latter a lesion made its appearance at the point where the needle penetrated the skin, and in only one did a lesion appear in the sub-

TABLE III.
Relation of Inflammatory Reactions of Various Types and Age to a Superimposed Syphilitic Infection.

	Rabbit No.	Syphilitic lesion.	Incubation period. <i>days</i>
Group A (unbroken skin).	24	None.*	
	25	" *	
	26	"	
	27	"	
Group B (subcutaneous inoculation).	28	None.*	
	29	Two chancres.	34
	30	None.†	
	31	Chancre.	34
Group C (shaved skin).	32	Chancre.	30
	33	"	26
	34	"	34
	35	"	26
Average.....			29
Group D (preliminary coal tar dermatitis).	36	Numerous papules.	34
	37	" "	34
	38	Few "	34
	39	Numerous "	38
	40	Several "	34
	41	" "	34
42	Numerous "	34	
Average.....			34.5
Group E (fresh wounds).	43	Chancre.	34
	44	"	38
	45	"	38
	46	"	30
	47	"	38
48	"	38	
Average.....			36
Group F (14 day wounds).	49	Chancre.	30
	50	"	22
	51	"	26
	52	"	26
	53	"	21
54	"	20	
Average.....			24

* Lymph node transfer to normal animals was performed and in each instance was negative.

† Lymph node transfer to normal animals was positive.



TEXT-FIG. 1. Experiment 3. Tracing of chancres of rabbits, taken at time of maximum size, showing greater size attained by those developing in old wounds (Group F) as contrasted with those developing in fresh wounds (Group E). The solid black area indicates the exact outline of the area of necrosis. The black line indicates the outer limit of the zone of induration. Reduced to 1/3 original size.

of the epithelial cells of the skin. In sections from such an area there is to be seen marked thickening of the epidermis occasioned by a remarkable hyperplasia of the basal cells. One sees down growths

cutaneous tissue where the bulk of the inoculum was deposited. In one of the animals in this group in which no local lesion developed, infection, nevertheless, occurred as shown by positive lymph node inoculation in normal rabbits. In all of the animals (Group C) in which a scarified area was inoculated characteristic chancres developed. In the case of the animals with fresh wounds (Group E) chancres developed ultimately in every instance, the average incubation period being 36 days, and in the animals with 14 day wounds (Group F) chancres also developed in each instance, the average incubation period in this group being 24 days. In the latter group the maximum size attained by these chancres was appreciably greater than that attained by the chancres developing in the fresh wounds, as is shown in Text-fig. 1. In the case of the animals in which a dermatitis was produced prior to inoculation by the application of coal tar (Group D), typical syphilitic lesions resulted in each instance. In many of them the lesions were exceedingly numerous and were distributed over the entire inoculated area. They were small, indurated papules, violet-red in color, at first discrete but later tending to coalesce and undergo superficial necrosis (Figs. 4 and 5).

Coal tar applied to the skin of a rabbit in the manner outlined produces, as is well known, a dermatitis in which a prominent feature of the reaction is the proliferation

of epithelium and proliferation of cells lining the hair follicles. There are some edema and dilatation of the capillaries and an increase in the wandering cells and fixed tissue cells, but the most striking feature is the growth of epithelial cells.

DISCUSSION.

The experiments would indicate that a non-specific inflammatory reaction involving the skin and subcutaneous tissue favors the initiation and development of a primary syphilitic lesion in the rabbit. Even comparatively slight injuries to the skin, such as are produced by shaving off the epithelium, constitute a favorable nidus for the survival and multiplication of *Treponema pallidum* when applied in large numbers. It is, of course, impossible to determine the extent to which the injury alone is responsible for the establishment of syphilitic infection, as opposed to the part played by the local inflammatory reaction, since with every injury there occurs a greater or less reaction upon the part of the body. We are inclined to attribute considerable importance to the latter factor, since in general our experiments have shown that the more advanced the non-specific inflammatory reaction at the time of inoculation, the sooner and the more regularly does the primary syphilitic lesion appear and the greater size does it ultimately attain. The most favorable time for inoculation of a wound was found to be that when the wound was granulating nicely and was well on the way toward healing.

The reason for the apparently greater susceptibility of granulation tissue to syphilitic infection is not entirely clear. One explanation which should be considered is the possibility that phagocytosis is less extensive in a granulating wound than in a fresh one.

Not much is known concerning the degree to which phagocytosis operates in syphilitic lesions. Ehrmann⁵ claims to have found histological evidence in chancres of phagocytosis of treponemata by the large wandering cells. Levaditi⁶ has seen in sections of the lungs of congenitally syphilitic children what he regards as instances of phagocytosis of treponemata by the macrophages and polymorphonuclear leucocytes. On the other hand, Zinsser⁷ as a result of his experiments

⁵ Ehrmann, S., *Wien. klin. Woch.*, 1906, xix, 828.

⁶ Levaditi, C., *Ann. Inst. Pasteur*, 1906, xx, 41.

⁷ Zinsser, H., *Infection and resistance*, New York, 2nd edition revised, 1918, 515.

with mice is very doubtful if phagocytosis of treponemata actually occurs before the death of the organisms themselves. If phagocytosis of treponemata by the macrophages is a prominent factor in reaction to syphilitic infection and phagocytosis by the polymorphonuclear leucocytes is rare, then we might expect it to be less common in the fresh wounds in which the latter cells predominate, and hence we should expect the greatest number of takes in fresh wounds, which is the reverse of what has been obtained in our experiments. It would appear that before phagocytosis can be invoked as the sole explanation for the results we have obtained, much more information as to the extent to which it occurs in syphilitic infection and the type of cell which participates in the process must be forthcoming.

Some observations of Levaditi and Yamanouchi⁸ have a bearing perhaps upon the phenomenon which we have described, and should be mentioned at this point. These investigators inoculated the anterior chamber of the eyes of a series of rabbits with fragments of syphilitic corneas and studied the eyes at varying intervals after inoculation to determine their treponemal content. They found that the treponemata in the inoculated fragment rapidly degenerated for the most part and there was no evidence of immediate multiplication of the organisms in the aqueous humor. Later, when organisation had set in around the inoculated fragment the organisms were found to be exceedingly numerous in the new tissue surrounding the fragment, and particularly in those regions in which there was regeneration of epithelium. These authors are convinced that the treponemata do not begin to multiply as soon as they are inoculated but that growth is retarded until the inflammatory reaction provides an abundant food supply. They would attribute the long incubation period in syphilis to delayed growth of the treponemata occasioned by lack of suitable nutritive material. It is evident that one might just as readily explain their results by assuming that the inflammatory reaction is the product of the growth of treponemata and is delayed by reason of the initial retardation of growth of these organisms. They did not perform the crucial experiment of inoculating a region which was already the site of an inflammatory process. Nevertheless, the phenomenon observed by them appears to be analogous to that which we have described, and it is possible that the explanation they offer may serve to explain both phenomena. In other words, the apparent greater

⁸ Levaditi, C., and Yamanouchi, T., *Compt. rend. Soc. biol.*, 1908, lxiv, 313.

susceptibility of granulating wounds to syphilitic infection may be due to the fact that the non-specific inflammatory reaction acts to provide an increased food supply for the treponemata.

Histological study of such wounds has seemed to us to bear out such a possibility. In granulating wounds, as is well known, the reparative processes predominate, and we have been struck by the evidence of cell growth that one sees in sections of such wounds. Large numbers of new fibroblasts are being formed and there is marked hyperplasia of the basal cells of the epithelium at the wound margins. It is evident that conditions are extremely favorable to cell growth. Not only may substances be present which act as a stimulus to the growth of the fixed connective tissue cells ("trephones" of Carrel,⁹ "archusia" of Burrows¹⁰), but in addition there must be an abundance of foodstuffs necessary to the new cell formation. It is possible that the same factors which bring about or contribute to active cell proliferation in a wound may also exert a favorable effect upon the growth of the treponemata. We do not wish to concern ourselves at this point with the nature of these factors; whether they represent hypothetical growth-promoting substances derived from wandering cells, or an excess of nutritive substances from the blood, or metabolic products of cell nutrition, is beside the point just now. We wish merely to point out that there may be a similarity in the response of both body cells and *Treponema pallidum* to the factors which stimulate cell growth in healing wounds; in other words, that anything which favors the growth of the former may also favor the survival and growth of the latter.

Such an hypothesis suggests the possibility of a close agreement between the metabolism of the human cell and that of *Treponema pallidum*. Theobald Smith¹¹ has already suggested such a possibility, but nothing definite is known about the metabolism of *Treponema pallidum*. Its nutritional requirements are as yet very imperfectly understood. It is known to be a fastidious organism, one that will not grow upon the ordinary laboratory media, yet will survive for decades within the animal body. Despite the close proximity to

⁹ Carrel, A., *J. Am. Med. Assn.*, 1924, lxxxii, 255.

¹⁰ Burrows, M. T., *J. Med. Research*, 1923-24, xlv, 615.

¹¹ Smith, T., *J. Am. Med. Assn.*, 1913, lx, 1591.

living cells it finds sufficient food to maintain life and insure pathogenicity. From this well known fact it is but a step to the assumption that some of the factors which operate in the human body to bring about rapid cell growth may exert a favorable effect upon the growth of the treponemata.

As a corollary to this hypothesis those portions of the body where there is active cell growth should be particularly favorable sites for the growth of *Treponema pallidum*. In this connection it may be pointed out that the testicle is a particularly favorable site for inoculation with these microorganisms and there is probably no portion of the body in which there is a greater formation of new cells.

The recent work on vaccine virus and its relation to actively growing cells of ectodermal origin is worthy of mention at this point, because of its possible bearing upon the hypothesis that we have offered.

Levaditi and Nicolau¹² showed that by simple epilation and subsequent intravenous inoculation of a rabbit with vaccine virus, it was possible to bring about localisation of the virus in the hair follicles from which the hairs had been removed. These investigators found, furthermore, that within 24 hours after the removal of the hair in the normal animal there was histological evidence of proliferation of the epithelial cells at the base of the hair follicle, with frequent mitotic figures. This process lasts 3 to 4 days. In the infected animal the same proliferation of epithelial cells at the base of the hair follicle is seen at the end of 24 hours, but the proliferative process lasts longer than in the normal animal and sections made at a later time show that the area where there is active proliferation of epithelial cells is also the site of characteristic changes produced by the vaccine virus.

Levaditi and Nicolau¹³ also found that when epitheliomas of rats and mice are inoculated directly with vaccine virus the virus establishes itself in the tumor cells, brings about an increase in size of the tumor, and apparently vegetates and even multiplies there. Furthermore, if the virus is inoculated intravenously in rats or mice with epitheliomas it localises in the tumor cells. Similar relations were not found to hold for sarcomas, however. On the basis of these observations it would appear that vaccine virus has a particular affinity for actively growing cells of ectodermal origin. It may not be amiss to point out that vaccine virus and *Treponema pallidum* are alike in one respect; namely, their ability to multiply in the testes of suitable animals. It is possible that both may also possess a particular affinity for actively growing cells of ectodermal origin.

¹² Levaditi, C., and Nicolau, S., *Compt. rend. Soc. biol.*, 1922, lxxxvi, 986.

¹³ Levaditi, C., and Nicolau, S., *Ann. Inst. Pasteur*, 1923, xxxvii, 443.

The hypothesis that we have offered for the phenomenon which we have described, namely that active proliferation on the part of certain cells of the body, particularly the fixed connective tissue cells and the epithelial cells, is directly associated with multiplication of *Treponema pallidum*, may be invoked to explain certain phenomena observed in the course of syphilitic infection in human beings. It may explain why treponemata are so extremely numerous in the human syphilitic fetus, since an enormous cellular proliferation is taking place in the latter. Furthermore, it is conceivable that the phenomenon which we have described may be related to the well known fact that trauma in a syphilitic individual predisposes to the development of a syphilitic lesion at the site of injury.

Whatever the explanation of the phenomenon, there can be no doubt that a non-specific inflammatory reaction in the rabbit furnishes a particularly favorable site for the survival and growth of virulent strains of *Treponema pallidum*; and for the development of a characteristic chancre, granulating wounds are especially favorable. At times more than one chancre may develop in such a wound (Fig. 6) and it is even possible to produce chancres which in their form correspond to a predetermined pattern (Fig. 7). The finding discloses a new method for producing syphilis in the rabbit that has certain manifest advantages from an experimental standpoint. It would be of interest to determine to what extent this method may be applied to the problem of transmission of other infectious diseases to laboratory animals.

SUMMARY.

Rabbits can be successfully infected with syphilis by the inoculation of surface wounds with virulent strains of *Treponema pallidum*. Old granulating wounds in these animals constitute a particularly favorable terrain for syphilis inoculation, the chancre developing relatively soon and attaining a greater size than the lesion occurring on the basis of a fresh wound. Coal tar dermatitis renders the skin susceptible to infection with *Treponema pallidum*.

The possible reasons for these phenomena are discussed and an hypothesis to account for them is advanced.

EXPLANATION OF PLATES.

PLATE 19.

FIG. 1. Side view of beginning of chancre developing in 16 day wounds, taken 27 days after inoculation and 5 days after appearance of lesion.

FIGS. 2 and 3. Same lesion 25 days later.

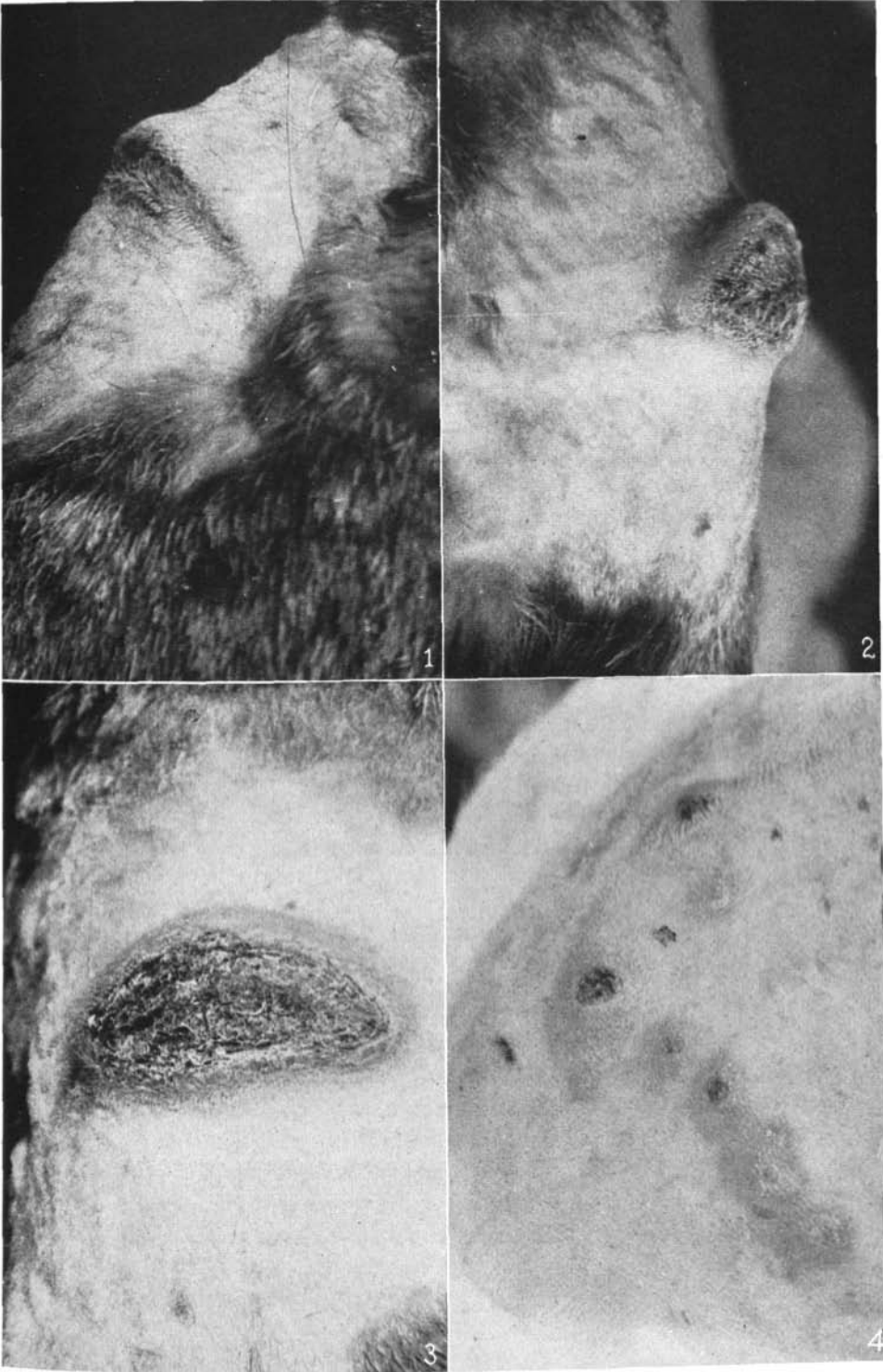
FIG. 4. Syphilitic papules produced by inoculating skin which was previously the seat of a dermatitis produced by coal tar. Taken 48 days after inoculation and 14 days after the appearance of lesion.

PLATE 20.

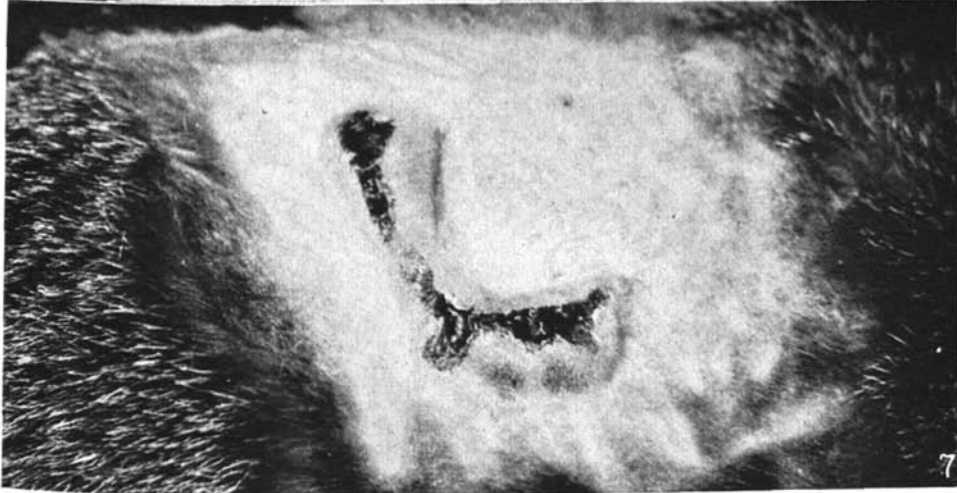
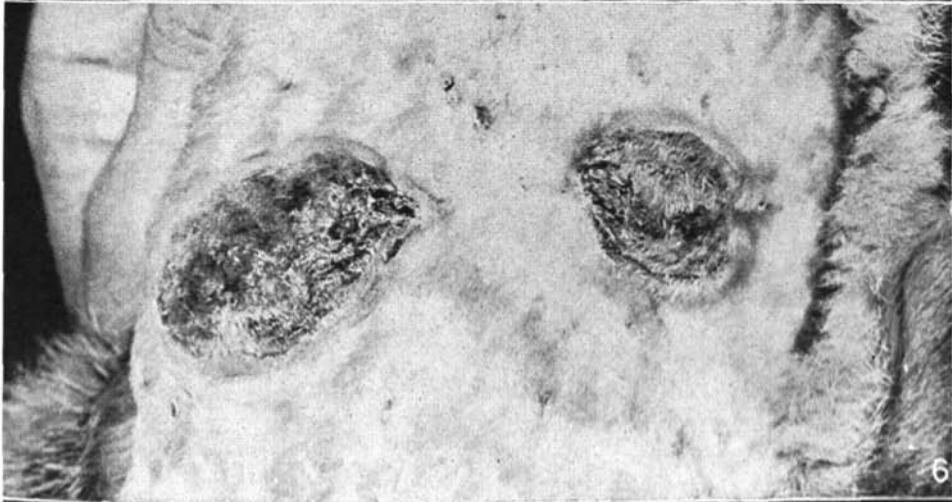
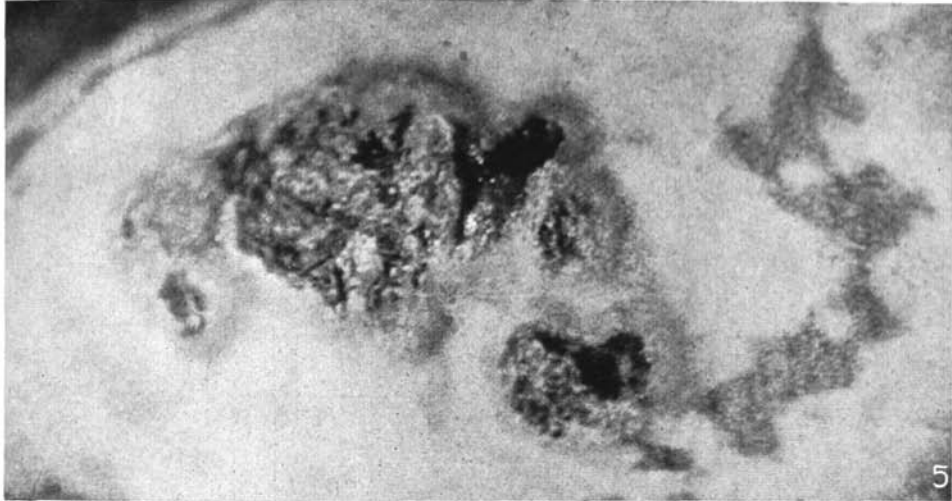
FIG. 5. Confluent syphilitic papules produced on skin the seat of coal tar dermatitis. Taken 63 days after inoculation.

FIG. 6. Two chancres produced by inoculating a single granulating wound.

FIG. 7. Result of attempt to produce an L-shaped chancre by inoculating an L-shaped granulating wound.



(Chesney and Kemp: Experimental syphilis. II.)



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