The histology of vaccinia by S. Monckton Copeman and Gustav Mann, being Appendix C to the Report of the Medical Officer (to H.M. Government)

(620)

With Dr. Copeman's kind regards.

REPORT

OF THE

MEDICAL OFFICER.

APPENDIX C.

The Histology of Vaccinia.

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This is I believe the only copy of this report!
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(620)

APPENDIX C.

THE HISTOLOGY OF VACCINIA; by S. MONCKTON COPEMAN M.A., M.D., F.R.C.P., and GUSTAV MANN, M.D., B.Sc.

PART I.—INTRODUCTION; by DR. COPEMAN.

It is a somewhat remarkable fact that although, for a century past, practically every medical man has had more or less opportunity of becoming acquainted with the clinical appearances resulting on the inoculation of the human subject with cow-pox, or vaccinia, comparatively little attention has been paid to the minute anatomy of the various stages of the eruption typical of this affection. I have long been desirous that this omission should be repaired, being convinced that an accurate knowledge of the histological changes involved could hardly fail to throw some further light on the pathology of vaccinia, concerning which, even at the present time, so much divergence of opinion exists. But the time at my disposal has proved insufficient to enable me to carry through the necessary investigations unassisted. Several years ago I suggested to Mr. Stanley Kent that he should undertake, with me, a detailed investigation of the histology of the vaccine vesicle, and for this purpose I provided him with a complete series of the necessary specimens of skin from a previously vaccinated calf. But, for various reasons, he has been unable to proceed further with the research than sufficed to enable him to read at the Bristol meeting (1894) of the British Medical Association a short preliminary paper on the subject, an abstract of which was subsequently published in the Journal of the Association.* Under these circumstances I asked Dr. Mann, who I found was also interested in the subject, to undertake the histological examination of material which I collected for the purpose. The scope of the work and the manner of carrying it out, together with the probable interpretation of the results obtained, have been matters of frequent discussion between us. But the actual histological work (the account of which forms the chief bulk of the paper, and nearly all that is interesting in it) was performed by Dr. Mann alone.

We desired to embrace, in our investigation, microscopic examination of the skin throughout the sequence of events following on vaccination, from the time of implantation of the virus beneath the surface of the epidermis up to that at which the vesicle attains maturity and onwards through the phase of desiccation of the pustule and the subsequent healing of the skin beneath the "crust."

This being so, it was obviously impossible to obtain the necessary material from the human subject, although Dr. Cory, by vaccinating, as occasion served, the supernumerary digits of such children as came under his observation with this abnormality and subsequently removing the vaccinated digit, had been enabled, some years ago, to investigate to some extent the changes occurring as the result of vaccination in human skin.

* British Medical Journal, Vol. II., 1894, page 633.

For the purpose, therefore, of making sure of obtaining specimens of skin at the precise periods required, the material, on which the results of the present research are based, was obtained from the calf, small pieces of skin being excised from a previously vaccinated area at such subsequent intervals of time as had been determined on. For this purpose deep anæsthesia was found to be unnecessary, the operation being of momentary duration only. In order to avoid, as far as might be, fallacy arising from the slightly differing rate at which the process of vaccination reaches its height in different calves and under varying circumstances, the influence of which are at present but imperfectly understood, several sets of specimens were procured.

In all instances the vaccination was carried out by means of linear incisions on the abdomen after the manner now ordinarily adopted at the Government Animal Vaccination Establishment. Each piece of skin, excised so as to include little else than the tissue immediately bordering on the line of incision, was, on removal, at once divided transversely into two portions, one of which was placed in absolute alcohol, the other in a watery solution of picric acid and corrosive sublimate. These small specimens of skin were then wrapped in absorbent cotton wool, placed each separately in a tiny wide-mouthed stoppered bottle, carefully packed, and despatched to Dr. Mann, at the Physiological Laboratory, Oxford.

In order to assist the reader in following the minuter histological details set out in Part II. of this paper it may be well here to give a brief account of the macroscopic and coarser microscopic appearances which are to be observed in the skin during the local development of the vaccine vesicle. It must, however, be borne in mind that in the calf the whole process of vaccination runs a distinctly shorter course than in the human being, a fact that is probably in some measure dependent on the normally higher body temperature of the calf as compared with that of man. Thus in the human subject the vaccine vesicle ordinarily attains maturity on the *8th day* [(24 hours × 7) = 168 hours], while in the calf the vesicle will have attained a similar stage of development on the *6th day* [(24 hours × 5) = 120 hours].

Within an hour after vaccination, more particularly in the calf, the skin immediately bordering on the inoculation wound not infrequently becomes somewhat raised owing to a transient local urticaria. This, however, rapidly passes off, and, in the human subject, practically no further change becomes obvious before the third day after the insertion of vaccine lymph, by which time a small inflamed spot or "papule" may usually be observed at the point where the vaccination was performed. Next day this spot appears more florid, and on passing the point of the finger over it a certain degree of hardness and swelling is perceptible. By the fifth day the papule develops into a small pale vesicle. This vesicle has a milky white colour, it is depressed in the centre, and its edges are distinctly elevated above the level of the surrounding skin. As yet the vesicle has no inflammatory zone around it.

For the next two days the vesicle increases in size; assuming, if the vaccination was performed by the method of puncture, a circular form; if done by an incision, an oval shape. But in both

cases the margin is regular and well defined. About the eighth day an inflammatory zone of a bright red colour, termed the "areola," begins to appear around the base of the vesicle; this increases in extent for two or perhaps three days more, by which time it may extend for about a couple of inches from the vesicle. The latter still retains its concave appearance, but a crust of a brownish colour will have commenced to form in the centre. By about the eleventh day the vesicle has attained its greatest magnitude, and the surrounding inflammation begins to abate. The fluid contained in the vesicle, or "pustule" as it is now called, which before was thin and transparent, becomes more viscid and somewhat turbid. After this period the whole becomes quickly converted into a smooth shining dry crust of a dark brownish colour. This crust, unless forcibly removed, will adhere for a week or more and then fall off, leaving the skin beneath apparently sound, but livid for a time, and afterwards more or less permanently scarred.

During the evolution of the local changes which result from the insertion of vaccine lymph beneath the surface of the skin it is possible, as previously mentioned, to recognise three more or less definite stages of papule, vesicle, and pustule.

The same statement holds good with reference to the eruption of small-pox, whether this be local, *i.e.*, due to intentional inoculation of the virus, or general, as the result of casual infection.

In each instance the appearance of the first or *papular* stage is brought about by inflammatory reaction, causing an increase of intercellular fluid, together with concomitant increase in volume and number of epithelial cells, of the rete Malpighii more particularly. The papule gradually becomes enlarged by a circumf rential extension of the same process, and owing to further changes in the cells first affected, vacuoles arise in the central portion of the papule, by the extension of which this ultimately becomes a vesicle.

The *vesicle* is a multi-locular structure, the dissepiments, by means of which its interior is divided up, being formed from the thinned and extended remains of the original epithelial cells. Owing to the fact that the process of vacuolation increases, for a time, more extensively at the advancing edge of the vesicle, the central portion remains somewhat less elevated, thus giving rise to the appearance termed umbilication.

At a somewhat early stage of the process an outflow of leucocytes takes place towards the point of injury. In time each blood vessel becomes the centre of an aggregation of leucocytes, which by the rapid increase in their numbers eventually transform the originally clear inflammatory exudation into a purulent fluid. The vesicle is said now to have become converted into a *pustule*.

By the thinning and ultimate rupture of its trabeculæ the pustule finally becomes unilocular. The turbid fluid contained in it now gradually dries up, and, together with the necrosed remains of epidermal cells, takes part in the formation of the *crust*, which, under the microscope, appears as a homogeneous mass very deeply coloured by the ordinary stains.

Meanwhile a regeneration goes on underneath the crust, the new epidermis being formed by an ingrowth from the surrounding *stratum lucidum*. The extent to which the cutis vera has been involved determines the depth of the resulting scar

APPENDIX C.

On the Histology of Vaccinia; by Drs. Copeman and Mann.

PART II.—HISTOLOGY; Plates XX.–XXXIII.;

By DR. GUSTAV MANN

(From the Physiological Laboratory, Oxford).

Methods Employed in the Present Research.

The pieces of skin, as soon as excised, were placed for 24 hours into either absolute alcohol or picro-corrosive solution, this latter consisting of one part of saturated $HgCl_2$ in $\frac{3}{4}$ per cent. NaCl (= 10 per cent. solution of $HgCl_2$) mixed with three parts of a saturated watery solution of picric acid (solubility of picric acid = 0·6 per cent.).

The specimens were passed in the usual way through chloroform and paraffin. Sections were cut varying in thickness from 2·5–25μ, and were fixed to slides by a special albumen method;* and also, without any fixative, by Gulland's method. This latter precaution was taken to meet the possible objection that the albumen on the slide might have given rise to appearances which have nothing to do with vaccination. I am, however, able to state that absolutely no difference can be seen between sections fixed by one or the other method, while by the use of albumen one can make sure of sections adhering to the slide during the prolonged staining with alkaline or acid solutions involved in certain of the methods adopted.

After the removal of the paraffin from the sections with xylol and absolute alcohol, they were first examined unstained, in the following media, which are arranged according to their refractive indices: water = 1·333. absolute alcohol = 1·361; glycerine and water equal parts = 1·397, chloroform = 1·449, glycerine = 1·456, Bergamot oil = 1·464, xylol = 1·497, Canada balsam = 1·535, metacinnamene with an equal weight of phenylthiocarbimide = 1·639. Phenylthiocarbimide (C_6H_5CNS) = 1·654, methylenediiodide (CH_2I_2) = 1·743$^{\alpha}$D. The three substances last mentioned were kindly suggested and given me by H. G. Madden.

Of these substances it is well always to use water, Bergamot oil, balsam and the metacinnamene-phenylthiocarbimide mixture. The last of these forms also a valuable addition to our list of mounting media, for which purpose it should be employed in conjunction with Bergamot oil, balsam and xylol balsam.

In addition to the investigation of tissue elements by examination in media with a higher or a lower refractive index than they possess themselves, recourse was had to staining reagents, and, again, sections were first examined in water to ascertain what elements give up their colour on being treated with alcohol.

It is difficult to arrange the dyes which were used, systematically, but the following list may serve as an indication of the line of research adopted:—

For histological, apart from bacteriological, purposes the chief methods employed were:—

A. Substantive ones (Bancroft), *i.e.*, without previous mordanting.

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* Anat. Anz. Bd. VIII., 1893, p. 442.

(1) Acid dyes :—

(a) Mann's biacid mixture of methylblue and eosin. Methylblue is the triphenylpara-rosanilintrisulfoacid ($C_{37}H_{26}N_3S_3O_9Na_3$).

1 per cent. methylblue in distilled water ...	35 c.c.
1 per cent. eosin in distilled water	45 c.c.
Distilled water	100 c.c.

Sections are left in this mixture for five to ten minutes, washed in water, dehydrated and mounted in balsam. This constitutes what may be termed the "short method." Or they are dealt with by the "long method," *i.e.*, are left in the stain 12–24 hours, then washed in distilled water, thoroughly dehydrated, and placed in a vessel containing absolute alcohol 30 c.c. to which, previously, five drops of a 1 per cent. solution of KOH in absolute alcohol have been added. When the sections have turned a reddish tint, the slide is washed with absolute alcohol to remove the alkaline alcohol, then rinsed in distilled water till differentiated. If the sections are not blue enough, a drop of acetic acid added to the water in which they are being rinsed will restore the colour.

(b) Picro-nigrosin, in a watery solution.

(c) Ehrlich's triacid mixture.

(d) Ehrlich's acid haematoxylin.

(2) Neutral dyes :—

(a) Ehrlich-Biondi mixture.

(b) Jenner's methyleneblue-eosin precipitate dissolved in pure methylic-alcohol (Merck). Stain for one hour. Rapidly wash in absolute alcohol and clear.

(c) Delafield's haematoxylin made with haematein. Two drops of this solution in 30 c.c. of distilled water. Stain for three to seven days.

(3) Alkaline dyes :—

Toluidinblue, thionin, methyleneblue (the polychrome methyleneblue of Unna is a mixture of methyleneblue and dimethylamin hydrochlorate or methylene violet and methylene red).

Methylviolet 6B was used in Kromayer's modification of Weigert's fibrin stain for the demonstration of the fibrils in epithelial cells.

B. Adjective methods used were :—

(1) M. Heidenhain's iron-alum haematoxylin.

(2) Rawitz' tannin and tartar emetic, followed by fuchsin, safranin, methylviolet, gentian-violet and smaragd-green.

(3) Wasielewski's mixture; fuchsin one part, potash alum three parts, water 100 parts, followed by ½ per cent. $K_2Cr_2O_7$ in 70 per cent. alcohol.

Special bacteriological methods used included the following :— Gram's original method, and its modifications according to Nicolle and Claudius; Löffler's methyleneblue (methyleneblue 0·5 parts, absolute alcohol 30 parts, and KOH (1 : 10,000 of water) 100 parts); Löffler's formula, but with 1 : 1,000 of KOH, *i.e.*, containing ten times the ordinary amount of alkali. Further, carbol-thionin and carbol methyleneblue were employed, also saturated solutions of toluindinblue, thionin and methyleneblue in 5 per cent. formaldehyde solution (calculated from formol, which is supposed to contain 40 per cent. formaldehyde, and

which requires to be carefully neutralized with magnesium—or some other carbonate to bind the free formic acid. (The use of caustic alkalies is inadmissable, as in their presence the formaldehyde polymerises.) The formaldehyde acts as a mordant.

Romanowski's stain was made according to Zethnow's directions, the greatest care being taken to exactly neutralize the normal soda solution.

Unna's polychrome methyleneblue followed by the use of a 33 per cent. solution of tannin.

Ziehl-Neelsen carbol fuchsin followed by alcohol containing 5 per cent. and 25 per cent. H_2SO_4.

Friedländer's and Möller's methods for spore-staining.

Of acid dyes alcoholic and watery, semi-, and completely saturated, solutions of acid fuchsin, with and without the addition of glacial acetic acid, have been employed.

Lastly, a number of combined stains were used, such as orcein followed by methylblue-eosin, or orcein followed by polychrome methyleneblue and tannin, or by formaldehyde toluindinblue.

In all, I have examined during the last three years over 1,500 slides, showing the structure of normal skin and of skin removed 1, 24, 48, 72, 96, 120, and 144 hours after vaccination. I beg to acknowledge the great service Miss Mabel FitzGerald rendered me in helping me to stain the preparations by the above methods.

Literature will be quoted only in as far as it bears directly on the results of observations.

For present purposes it is desirable in the first instance to give some description of the changes to be observed after vaccination in the skin under a low magnifying power, viz., 15 diameters, and then to detail successively the minute changes in the epidermis, dermis, and hypodermis.

On studying Fig. 1, Plate XX., which represents the appearances met with at about one hour after vaccination, one notices, externally, a clot which consists partly of the vaccine lymph applied and partly of serum exuded from the wound. The epidermis will be seen to have been completely divided, and throughout the section it is of uniform thickness, as also are the dermis and the hypodermis. During the next 48 hours the principal change first seen is one affecting the dermis (Fig. 2, Plate XX.), which is considerably swollen, the hair follicles having also increased considerably in size. The epithelial change is secondary in its appearance (Figs. 3 and 4, Plate XX.) and has resulted in a four to five-fold increase in thickness of this layer and a diminution in the affinity of the cells for both basic and acid dyes.

The vaccinated area is, at the end of 48 hours, forming a distinct papilla with a slight depression in the middle, due to the method of vaccination. Up till now there is very little leucocyte infiltration. After 72 hours (Fig. 5, Plate XXI.) the epidermis is much swollen, as is also the epidermal cell-structure of the hair follicles and sebaceous glands. The coagulum where vaccination took place is much larger than previously because of a necrotic change which has been spreading laterally, and also because of the increased leucocyte infiltration in the dermal layers. One feature common to Figs. 5, 6, 7, and 8 (Plates XXI. and XXII.) is the apparent one-sided development of the lesion, *i.e.*, if we take the centre of the clot as the point from which the affection is

spreading, then we find, probably because of the direction of the lymph flow, that one side of the skin undergoes its characteristic changes more rapidly and to a greater extent than the other.

During the first 48 hours the hypodermis is apparently normal, but in the course of the third day (Fig. 5, Plate XXI., representing changes after 72 hours) it begins to swell underneath the inoculated area. On the fourth and fifth days the œdema loses its local character, and is for this reason less evident in Figs. 6 and 7, Plates XXI. and XXII.

Whatever the nature of the virus may be, it does not give rise to much leucocyte infiltration during the first two days. Comparing Figs. 3 and 4, Plate XX. (48 hours), with Fig. 5, Plate XXI. (72 hours), there will be noted in the latter considerable leucocyte infiltration in the dermal layer, which during the fourth (Fig. 6, Plate XXI.) and fifth days (Fig. 7, Plate XXII.) becomes very marked, and on the sixth day (Fig. 8, Plate XXII.) results in the dermis having swollen to such an extent as to bulge into the hypoderm, and now the latter also becomes invaded, the thick collagenous bundles composing it being pressed apart.

The umbilicated appearance during the fourth and fifth days (Figs. 6 and 7, Plates XXI. and XXII.) appears, as Unna has suggested, to be mostly due to the original injury caused by the process of vaccination.

During the sixth day (Fig. 8, Plate XXII.) both the epidermis and the dermis are seen to undergo considerable necrotic changes. The size of the affected area can be ascertained without any difficulty, as all the photographs (Figs. 1–8, Plates XX.–XXII.) are taken to the same scale, viz., 15 diameters, and they are therefore directly comparable.

Minute Structure of the Epidermis.

To understand the changes which the epithelium undergoes as the result of inoculation with the virus of cowpox, it is necessary to first refer to the normal appearances.

In preparations fixed in alcohol and stained by Kromayer's method, or fixed in picro-corrosive solution and stained by Wasielewski's method, Ehrlich's tri-acid, or Jenner's fluid, the stratum corneum forms one-fifth of the total thickness of the epidermis, the stratum granulosum being represented by only one layer of cells, which latter are so few in number as not to be usually in contact with one another. The rete Malpighii is from three to four cells deep (*vide* later, Fig. 9, Plate XXIII.). The lowermost cells of this layer, *i.e.*, those in direct contact with the dermis, are columnar, with long, finger-like processes which enter the basal membrane (*vide* later). What is quite characteristic of all the cells in this layer is the presence of fibrils which in the lowest columnar cells run vertically and at the apices curve round into the neighbouring cells. The various cells are, in this way, firmly united to one another, the weakest spot being the area between the adjacent basal halves of the columnar cells where the bridging fibrils are very few in number.

On examining the fibrils lying between neighbouring cells (*i.e.*, the so-called "prickles") each is seen to have on its centre a minute granule, which was first noticed by Reinke. The inter-fibrillar matter in the intercellular region is formed by lymph

derived from the dermis; while the interfibrillar substance in the cell is a viscous cytoplasm, which in the rete Malpighii is fairly abundant, in the stratum granulosum undergoes a transformation into the basophil granules, and in the stratum corneum for the greater part, along with the fibrils, gives an eosinophilous reaction. That there exists a basophil substance seems to be demonstrated by specimens stained for one hour in Jenner's fluid, then rapidly washed in absolute alcohol and cleared in xylol, as by this method the stratum corneum is stained pale blue.

The nuclei in the rete Malpighii, when stained by either the short or the long methylblue-eosin method, stain a bright blue (80 per cent.) or brilliant red (20 per cent.). The blue nuclei usually show feebly developed nucleolar material.

Perinuclear spaces are very rare in normal skin.

Changes in the Epidermis due to Vaccination.

In all the pieces of vaccinated skin examined, the epidermis was for the most part completely divided along the whole track of inoculation. In any case the vaccine matter is brought directly into contact with both epidermis and dermis.

It is customary to distinguish in vaccinia three distinct histological appearances in the epidermis, viz., (1) Weigert's primary coagulation necrosis, due to the great virulence of the poison at the seat of lesion; (2) Unna's reticulating fibrinoid change, affecting chiefly the upper layers of the epidermis; (3) Unna's "ballooning colliquation," which is most evident in the lower strata of the rete malpighii.

1. *The primary coagulation necrosis* one would naturally expect to find close to the seat of infection; in the region where, during the early stages of infection, because of the temporary resistance offered by the healthy cells, and because of the want of circulation, the poison must accumulate. When the cells, therefore, once commence to fall victims to the irritant, they quickly succumb. This condition is best seen at the end of the second and commencement of the third day, close to the line of inoculation, but only one or two cells in each section are to be found which can be said to have degenerated without having passed through the typical changes immediately to be described.

It may be doubted, however, whether the explanation suggested is the whole explanation; for it is quite possible that the cells which have succumbed have done so in consequence of direct mechanical irritation, the result of the process of vaccination. The cells referred to do not show the typical fibrillar arrangement, but are smaller than normal, the cytoplasm is granular, amphophil, either collected centrally round the nucleus or occupying with the latter an excentric position, the remainder of the cell being empty and not traversed by fibrin threads.

2. *The reticulating fibrinoid change*, according to Unna, takes place thus:—Near the nucleus, or near the periphery of the cell, small vacuoles filled with fluid appear. These, becoming confluent, form a net which joins the nucleus to the cell wall. This net then undergoes a fibrinoid change, and has precipitated on it fibrin-granules from the intra-cellular fluid, which granules Renant supposed to be micrococci. The long preservation of the

nucleus and the cell wall with its prickles is stated by him to be characteristic of this change.

3. *The "ballooning colliquation"* of epidermal cells, according to Unna, is characterised (1) by the cytoplasm, as a whole, undergoing degenerative changes, there being thus no demarcation into a peripheral and central zone; (2) by the loss of the prickles and rounding off of the cells in the rete Malpighii; (3) by the amitotic division of the cell-nucleus giving rise to numerous nuclei; (4) by an increased plasticity of the cell which, adapting itself to its surroundings, will assume a globular, flattened, or pointed shape; (5) by an ultimate fibrinoid change affecting both the cytoplasm, or its remains, and the nuclei. In vaccinia complete degeneration usually results, while in varicella regeneration of these cells is possible.

Examination of the specimens shows that the earliest change is an increase in the diameter of the intercellular channels (*vide* Fig. 9, Plate XXIII.), but how this is brought about is doubtful. We certainly find evidence of œdema in the subjacent dermis, and might therefore suppose that the intercellular spaces become distended with lymph, which, being unable to escape on the surface, forces the cells apart, and thus renders the "prickles" much more evident. On the other hand a possible power of contraction inherent in the cells, and called forth by the action of the virus, must be taken into account. That during later stages this tendency does show itself will become evident. On studying Fig. 9, Plate XXIII., it will be noticed that the prickles are in reality the intercellular portions of fibrils coursing from cell to cell and uniting them to one another. Unna in his textbook has paid no attention to Ranvier's discovery of these fibrils, and speaks of the "homogeneous protoplasm of the younger cells," when describing the ballooning colliquation of varicella. In addition to these fibrils there is found in normal cells the interfibrillar cytoplasm which has a mesh-like arrangement, as is seen in Fig. 12a, Plate XXIV. In describing the epidermal changes, it is necessary to consider separately the fibrillar and the non-fibrillar elements, and also to realise that the latter can retract from the nucleus, although under normal conditions they are in contact with it. By the retraction of the non-fibrillar elements a perinuclear space is formed.

In tracing the changes which the cells in the upper layers of the rete Malpighii undergo under normal conditions, and those which they show under the action of the virus, a sharp distinction must be drawn between bodies formed inside the perinuclear space and those found outside it in the cytoplasm. In Fig. 9, Plate XXIII., the cytoplasm of the cells, No. 1, is shown in direct contact with the nucleus, while in No. 2 a perinuclear space has been formed. This latter appearance is normally found in the upper layer of the skin, and becomes especially well marked in those changes which accompany the formation of nails and hoofs, but although I have looked through the large collection of epidermal sections kindly lent me by Prof. Arthur Thomson, I failed to find any structures lying within the perinuclear sac. In the cell, No. 4, Fig. 9, a pointed body lies against the periphery of the nucleus, apparently not within a space, and in the cytoplasm is found an oval body looking like a diminutive nucleus. The significance of these bodies

is not known, but that element lying close to the nucleus may be a very early stage of Guarnieri's supposed parasite.

The various papers dealing with epithelial changes said to be due to the action of parasites may thus be summarised :—

In 1886–7, Van der Loeff, in three papers, described in vaccine lymph and in variola vera, "proteid" (protozoa) or amœba-like bodies.

L. Pfeiffer, 1887–91, arrives at the conclusion that the contagium is a parasite belonging to the class of sporozoa (Leuckart), and calls it *Monocystis epithelialis*. There cannot be any doubt, if one takes into consideration the figures accompanying the above papers, that both authors mistook epithelial cells for sporozoa, viz., those cells which Unna describes as having undergone a ballooning colliquation. This explanation of Unna's is fully confirmed by the results of the present research.

Entirely different structures were subsequently described in 1892 by Guarnieri as parasites. During the prepustular stages small granules the size of cocci are seen at the periphery of the lesion while more centrally they may reach in size one-half the diameter of cell nuclei. Usually they are placed at a certain distance from the nucleus but when lying close to it they are in most cases invaginated by it, owing to their plastic condition. On very rare occasions they may indent the nucleus. These later stages are supposed to represent a rhizopod, capable of amœboid movement, with an evident nucleus and capable of dividing "without any doubt" by fission and probably also by endogenous gymnospore formation. Guarnieri applied to his supposed parasite the name of *Cytoryctes vaccinæ* or *variolæ* for the reason that it hollows out the cytoplasm of the epidermal cells.

Ferroni and Massari in 1893 reinvestigated the subject in Grassi's laboratory, and all three agreed that appearances as described by Guarnieri could be obtained by the application of such substances as croton oil, osmic acid, iodine, or Indian ink, and therefore held that Guarnieri's bodies were partly nuclear derivatives and partly leucocytes.

Guarnieri repeated the experiments of the two authors just mentioned with negative results (1894) and was confirmed in this by L. Pfeiffer, who states that no purely chemical reagents will produce such uniform results as vaccine, leaving the nuclei at first unaffected. He then states that Guarnieri's bodies are young or early stages of the bodies previously (1887, *see* above) described by him under the name of *Monocystis epithelialis*.

Monti (1894) described bodies similar to those of Guarnieri in the rete Malpighii, but classes them under the group of Lobosi rather than true Protozoa. According to this observer the elements measure 2–3 μ in diameter.

Piana and Galli-Velerio (1894) and J. Jackson Clarke (1893–95) all accept Guarnieri's view as did also Ruffer and Plimmer in 1894 at the International Medical Congress in Rome, with this restriction, that they were not convinced of distinct spore-formation.

In 1895 Sicherer confirmed Guarnieri and L. Pfeiffer, and E. Pfeiffer confirmed J. Clarke's statement as to the peripheral processes of his coccidia, which were stated by him to penetrate into the clear zone surrounding them, and even to pass through it.

He does not agree with the conclusions of Guarnieri and L. Pfeiffer, nor do his researches with glycerine, croton oil, osmic acid, or silver nitrate, confirm Ferroni and Massari.

The three most recent papers on this subject are by Jackson Clarke (1895), Wasielewski (1897) and Häckel (1898). Clark states that the variola parasites "find their most complete homology in those found in cancer and sarcoma." He distinguishes (a) hyaline globules 2–4 μ, (b) spherical bodies 5–7 μ, with a hyaline nucleus, finely granular protoplasm and one or more highly refractile granules, (c) ellipsoid bodies 7·5 μ, with two small nuclei, (d) amoeboid bodies 7·5 μ in their largest diameter, but with no distinct nucleus, (e) spheres about 8 μ, with pseudopodia, which can be fixed by a temperature of 20°C, and containing one or more large hyaline nuclei, (f) ovoid bodies 3–5 μ enclosed in a capsule possessing a fine opening at the pointed end. a, b, and c, usually occur free, occasionally in lymph cells or in the deep cells of the rete malpighii, d-bodies are always free, while "e" are partly free and partly found between epidermal cells; f elements are found most frequently in the middle layers of the epidermis. His Fig. 7 shows an enormous "parasite" nearly filling the whole of the epithelial cell, with peripheral granules, and a central fragmented nucleus, and his Fig. 12 free globular "parasites" with peripheral granules.

Wasielewski, a pupil of L. Pfeiffer, worked on the rabbit's cornea, fixed preferably in picro-corrosive solution, stained in alum-fuchsin, and differentiated by bichromate-alcohol. He also is an upholder of the protozoan theory and figures the smallest granular "parasite" at the periphery of the lesion and the largest ones near the point of inoculation. He especially points out that the bodies in question always lie in the perinuclear space. The nucleus at first spherical, may become indented by 1–3 bodies, which may either by a degenerative change give rise to ringlike structures encircling the apparently normal nucleus, or they may multiply and form spherical bodies with red granules at their periphery which lie in a concavity of the nucleus.

The most thorough and apparently unbiassed work which has been undertaken on this subject is that of Armand Häckel.

According to this observer the results obtained by inoculating the cornea of rabbits, show that Guarnieri's "parasites" give the following micro-chemical reactions:—

1. Distilled water has no action.

2. Potassium iodide stains them in the same manner as other cell constituents.

3. With saturated NaCl, the bodies and nuclei disappear.

4. Very dilute KOH causes both nuclei and bodies to disappear, and subsequent application of acetic acid does not restore them.

5. A one per cent. sodium carbonate solution destroys all detail in the cells, the nucleoli are no longer visible, and the bodies with their envelopes disappear. Subsequent treatment with acetic acid leads to the reappearance of the nuclei and bodies.

6. Five per cent. acetic acid renders the previously invisible small spherical bodies very evident, while the granules and droplets round the larger bodies disappear.

7. Flemming's solution renders the small bodies and the nuclear chromatin very distinct, while the protoplasmic granules round the larger cells disappear. The bodies are surrounded by a cleft.

8. One-half per cent. Na Cl saturated with $Hg Cl_2$ shows spaces round the bodies and nuclei in a few cells only. The same solution with the addition of acetic acid renders the bodies much more distinct and many pericorpuscular and perinuclear spaces become visible.

9. One per cent. osmic acid occasionally shows nucleoli in the epithelial cells, whether the bodies are present or not.

10. None of the reagents mentioned above will cause contraction in those bodies which have an amœboid shape.

11. With Biondi stain, Guarnieri's bodies stain blue and the leucocyte nuclei green, while with iodine green-fuchsin mixture, the former stain red while the latter stain blue, as do the nuclei of epithelial cells.

Häckel divides the appearances met with in vaccinated areas into several groups :—

A. Homogeneous bodies varying in size from those which are just visible, to others having a diameter of 3μ. They are usually found in the perinuclear cleft but occasionally lie in the cellplasm, and, if there, they are usually surrounded by a clear zone. The older the cell and the more cramped in position, the deeper the nuclear pocket in which the bodies lie.

B. Elements similar in position to those already described showing a distinct differentiation into a central blue and a peripheral red zone, the line of demarcation between the two being more or less sharp. The erythrophil external portion may undergo a granular or threadlike degeneration.

C. Similar to B, but the erythrophil granules are usually very regularly arranged and reach occasionally the size of granules A. These forms resemble the "daisies" of Guarnieri.

D. Spheroidal bodies surrounded by a broad clear zone, with threads extending through the latter into the cytoplasm (Clarke's and E. Pfeiffer's peripheral processes of coccidia). Fine red granules may be seen along the course of the fibrils, especially in cells lying towards the centre of the lesion.

E. Demilunes, spindles, &c., which appear later than the spheroidal forms, and always lie close to the nucleus.

F. Triangular or pyramidal forms which are very rare.

G. Lobular forms looking like blue amœbæ with red granules.

All the forms above mentioned are derived from the bodies first described under "A". The original small cyanophil body by imbibition of fluid begins to swell, and simultaneously the blue colour becomes violet. In addition to this, infiltration changes occur, affecting the periphery of the mass, and leading to the formation of erythrophil granules. Inasmuch as many granules protrude from the margin, the latter appears corroded. instead of only the periphery being affected by the imbibition one finds occasionally the vaccine body has undergone this hyaline change in toto. What is significant is that while the body shows the signs of hyaline degeneration, neither the cytoplasm nor the nucleus appear to suffer directly by these changes. Apart from

the hyaline degeneration one may find a vacuolation of the vaccine corpuscles which leads to the formation of, at first, coarse, then fine trabeculæ and ultimately fine granules. The early stages in this transformation induced Guarnieri to speak of filamentous forms of protozoa.

In addition to degenerative changes in the vaccine bodies, cells as a whole may undergo chromatoplasmolysis and form spheroidal bodies which Clarke mistook for the formation of sporogoniæ (with and without chromatin) and spores.

Häckel therefore considers Guarnieri's bodies not to be parasites, but to result from a peculiar transformation of a portion of the cellplasm due to the specific stimulus of the vaccine virus.

Three other suggestions which have been made are these: Babes (1894 International Medical Congress, Rome) referred to a possible nucleolar origin; Leoni (ibid) supposed Guarnieri's bodies to represent necrotic parts of the nucleus; and Salmon, working in Metschnikoff's laboratory, holds that they represent the hyperchromatic residue of nuclein derived from the nucleus of leucocytes.

Examination of our own specimens shows that after the preliminary dilatation of the intercellular lymph channels (Fig. 9, Plate XXIII.) the vaccine virus produces definite changes in the different layers of the epidermis, which will be described from below upwards. The general effect after 48–60 hours is seen in Fig. 20, Plate XXVII. Those cells which lie in direct contact with the dermis and belong to either the skin proper or to the root-sheaths of the hairs, are affected later than those lying in the middle layers of the rete Malpighii. This difference is due probably to the lowermost cells being more vigorous, as they contain a greater quantity of nonfibrillar cytoplasm, and also to the fact that pressure being exerted on them from all sides, will tend to prevent their increase in size. Already at an early period, e.g., 48 hours after vaccination in animals which have reacted well, the basal cells have increased from 13–15μ in length to 30–35μ; their nuclei showing a corresponding increase from 10μ to 13·5μ. The subsequent changes are not the same for all cells, for a certain number cease to react further and soon become atrophic owing to the pressure exerted on them by their neighbours. The remaining cells either undergo a reticular change which will be described later in connexion with the middle layer of cells or they give rise to what Unna has termed "balloons." The latter are formed (*vide* Fig. 14, Plate XXV., representing a section 72 hours after vaccination) by the epidermis first breaking up into a number of strands of epithelial cells, which appear as pillars surrounded by lymph, many of which, later on, because of a still greater accumulation of lymph, are torn across. There are thus formed *bullæ*, separating a superficial set of epidermal cells, from a deep set, the latter being still in contact with the dermis. Owing to this strain on the epidermal cells the intercellular bridges are torn across, and cells are liberated in groups of from one to ten. Amongst the larger groups there are some cells which are more vigorous and which for a time appear to thrive, while others are undergoing atrophic and reticular alterations.

The epithelial cells in addition to being affected as a whole, show also characteristic changes in their interior. At the early period, when the epidermal cells have commenced to increase in size, one or two small bodies appear at the poles of the cell (Fig. 12 k, Plate XXIV.). This occurrence bears out Häckel's observation on the cornea. The perinuclear space is not well marked, but there cannot be any doubt of the fact that these small bodies lie close to the nucleus within a clear space. Although hundreds of serial sections have been examined with the hope of tracing the origin of the granules, this has not been satisfactorily accomplished. When they first appear they measure about 0·5 μ in diameter, but soon they rapidly increase in size to 2μ (Fig. 12 l, Plate XXIV.): if there are two bodies present, the larger one is always found on the side nearest the surface.* The structure in question is one of the early stages of Guarnieri's supposed parasite.

The lowermost epidermal cells have a marked tendency towards nuclear proliferation, which expresses itself in one of the following three types:—

A. If the cell has become a "balloon," *i.e.*, if it is isolated, it may continue to grow till it reaches five times the normal diameter (Fig. 11, Plate XXIII.), the measurements for the cell No. 1 being 43μ × 75μ. What is characteristic of these cells is the feeble development of a perinuclear space, and the power of occasionally breaking up into a number of daughter cells (*vide* Fig. 13, Plate XXIV.) corresponding to the number of nuclei. This phenomenon has several analogues both in the vegetable and animal world.

The usual fate of these big cells is a reticular transformation, commencing round the nucleus and then spreading outwards. Fig. 11 represents the same cell stained by three different methods. The section in which it was first noticed was originally stained by Löffler's method for twelve hours, differentiated in alcohol, and mounted in balsam. This stain brought out the nuclei, and a number of basophil granules scattered throughout the cytoplasm. A reticular appearance could be seen on using a very small aperture of the diaphragm, but to make sure whether all the "prickles" had disappeared, the section was restrained in acid fuchsin and glacial acetic acid, and differentiated in bichromate alcohol, by which method the reticulum was revealed and a faintly stained granular appearance. To bring out the latter more fully, the section was treated by Möller's method for staining spores. The appearances which were seen by each of these methods were carefully recorded by the camera lucida, and ultimately combined into one picture. In the same Fig. 11 will be seen an epithelial cell which atrophied from the first, with a long collapsed nucleus (No. 2), and further, a binucleated cell (No. 3) with very regular reticulate transformation. The coarse fibrils extending upwards from the basal membrane and fixing the cell *in situ* are fibrin threads.

Belonging also to this type A, one finds, during the later stages of vaccination (120–144 hours), the appearances figured on Plate XXIV. (Figs. 12, o, q, and lower part of p, (p^1)). It is by no means certain

* Fig. 12 l is printed upside down.

as to what may be the correct interpretation of what is to be seen, but the following views may be suggested :—We are either dealing with leucocytes, which having entered the giant cells, are rendered inert by them through the deposit of a capsule, or the leucocyte, to protect itself, becomes surrounded by an envelope. This leucocyte hypothesis, however, does not seem so probable as the following theory :—As already pointed out above, the formation of daughter cells undoubtedly does occur, and the question arises, is it possible for that nucleus, which is the most vigorous, to surround itself by a capsule and thus to endeavour to safeguard itself. Appearances, such as are represented by these figures would seem to support the latter hypothesis, for we see an apparently normal nucleus surrounded by granular protoplasm, which communicates with the exterior by a definite narrow channel, while the greater part of the cell is surrounded by a thick capsule, showing a distinctly radial arrangement. In studying epithelial changes we should, as far as possible, endeavour to give a physiological explanation of the appearances met with, and not draw hasty conclusions as to their representing sporozoa. J. Clarke rightly points out that many cells appear similar to those found in cancer, but surely this is the best reason for not pinning one's faith to a parasite theory. On rare occasions I have succeeded in demonstrating, to my own satisfaction at any rate, that the capsule surrounding the cells gives the keratin reaction, and in one case the typical reaction of the granules met with in the stratum granulosum was present. We have, therefore, some grounds for the view that one or more of the daughter cells derived from a giant cell will subsequently pass through the same set of changes, which, under normal conditions, an epidermal cell undergoes in the course of its existence.

To refer once more to the giant cell in Fig. 11, Plate XXIII. :—On the right side of the perinuclear space will be seen a dark mass which represents the remains of a Guarnieri's body. The changes which this element undergoes will be detailed fully later on.

The ultimate fate of the cells belonging to the type A is shortly this :—The vacuolation and consequent reticulation commencing around the nucleus, spreads towards the periphery, the cytoplasm lying inside the vacuoles becomes gradually diminished, the cell loses its definite outline, leucocytes enter the body of the cell, and the nuclei derived from the original epidermal nucleus shrivel and lose their affinity for basic dyes, they become eosinophilous, and eventually give the same staining reactions as the reticulated cytoplasm. Unna calls the change a fibrinoid one, but the trabeculæ which are left never stain as deeply as true fibrin, and it is doubtful as to whether fibrin granules are really deposited on the surface of the cell network. Photographic views of these giant cells will be seen in Fig. 14, Plate XXV., and Fig. 16, Plate XXVI.

B. The second type of giant cells is characterised by the cell body undergoing condensation, due to the absorption of its fluid contents. This type approaches in its staining reactions much more closely to those of true fibrin. Here again one or more of the nuclei tend to surround themselves with cytoplasm and thus

form daughter cells within the confines of the mother cell. Fig. 12, Plate XXIV., represents an elongated cell with two atrophied nuclei at the apex (p^3), with four nuclei arranged in series in the middle (p^2) and a daughter cell enclosed in its capsule at the base (p^1). The four central nuclei are stained a brilliant red by Wasielewski's method, and possess a strong resemblance to Russell's "fuchsin bodies." They represent a typical change which nuclei in the uppermost layers of the rete Malpighii are apt to undergo on the fifth and sixth day after vaccination. Instead of passing from a vesicular into the usual contracted state, the nuclei become condensed into spherical masses, which, during the early periods, enclose a distinct nucleolus, but ultimately become homogeneous. The lowest cell in Fig. 12p., Plate XXIV., has become binucleated, and shows a smaller body towards the right, resembling in its staining reactions Guarnieri's bodies.

While the types A and B are formed, chiefly during the second, third, and fourth day, and always mark the height of the vaccine reaction, the next type is formed amongst apparently, as yet, quite healthy epithelium, principally during the fourth and fifth day.

C. The third type of giant cell differs from the two previous ones in being usually represented by a large number of vesicular nuclei free in a cavity among the surrounding cells. This type does not as a rule show any Guarnieri's bodies. What has become of the cell body of the original epidermal cell it is diffcult to say. The nuclei seem to have the power of division after the cytoplasm has completely disappeared. Should the epithelial cells which surround the giant cell undergo the typical change due to the vaccine virus which have been described above, the nuclei of type C are set free and soon undergo degeneration.

Unna has stated that the formation of the many nuclei in the epidermal cells takes place amitotically, but while this certainly seems to hold good for the majority of cases, there is no doubt that true mitosis also occurs.

It has already been pointed out that the epidermal cells in the upper part of the rete mucosum react differently to the vaccine virus from the lowest cells. The account of the changes to be given is based on the examination of sections fixed in picro-corrosive solution and stained at 30°C for 12 hours in Löffler's blue containing KOH in the proportion of 1 : 1,000. The solution employed was a fortnight old and contained therefore in addition to methyleneblue some methyleneviolet, due to the decomposition of the methyleneblue (Unna). Compare Fig. 12,A–E, Plate XXIV., seventy-two hours after vaccination. The sections were dehydrated with alcohol and mounted in xylol balsam.

Normal epithelium shows the nuclei stained deep blue, while the nonfibrillar cytoplasm is violet. The fibrillæ running through the cell remain unstained for which reason the individual cells stand out very sharply. The cytoplasm (Fig. 12A, Plate XXIV.) appears as a distinct sponge-work, the apertures or holes in which serve for the reception of the epithelial fibrillæ. In those cells close to the stratum granulosum where a contraction of the nucleus has taken place a delicate fibril seems to stretch

from either of the two poles of the nucleus and to extend into the cytoplasm. The nucleus at mid-focus seems to be drawn out into a fine point connected with these fibrils (Fig. 12B, Plate XXIV.)

The first change due to vaccination is an alteration in the regular arrangement of the cytoplasmic network close to the nucleus, some of the meshes becoming larger, and thus the appearance of vacuolation is produced. During this stage delicate strands of cytoplasm still connect the nucleus with the cell body proper (Fig. 12C, Plate XXIV.) Soon afterwards, during the stage of enlargement of the cell, the violet-coloured plasm assumes the appearance of a coarse meshwork, each corner of the meshes being markedly thickened (Fig. 12D[1] and D[2], Plate XXIV.) Still later the delicate strands joining the thickened nodal points tear across and thus give rise to little globules and rod-like structures. At this period the cells have a certain resemblance to the well-known picture of Nissl's granules in nerve cells (Fig. 12E, Plate XXIV.)

By the staining method employed the early stages in the formation of Guarnieri's bodies are not readily studied, but the later stages are well seen, as is also a peculiarity regarding the relative position of these bodies to the site of inoculation. It must be remembered that the calf is vaccinated by longitudinal incisions into the skin and that our sections were taken at right angles to this incision. Guarnieri's bodies are invariably situated in that half of the cell farthest away from the line of incision, thus in Fig. 14, Plate XXV., the cells all appear darker towards the right side of the photograph, the site of inoculation being on the left. On the parasitic theory this is very difficult to explain, but what proves definitely that the supposed parasites are not the cause of the change in the cells is the fact that the latter have undergone a complete vacuolar change in that half which is looking towards the point of inoculation, while that portion which contains Guarnieri's bodies is by far the more normal. In Figs. 12 F–I, Plate XXIV., five cells are delineated which exhibit this remarkable change. One half of each of these cells has lost all affinity for methylene-violet, while the other half stains deeply. Close to the site of inoculation the whole cell is unstained, or rather has a pale greenish tint resembling that of the fibrin threads. A little farther out the cell nuclei are still deeply coloured and in cells not quite as much affected, the degenerative change is spreading to that side of the cell farthest away from the inoculated area, and the supposed parasite is found to have undergone degeneration before the cytoplasm on which it is supposed to be feeding has completely disappeared.

It has been mentioned above that the early stages in the formation of Guarnieri's bodies connot be traced by this method, but that the later stages may be. It is well, however, here to point out that by the staining method employed, Guarnieri's bodies, as described by Häckel, stain exactly in the same way as the nonfibrillar cytoplasm.

If one studies the early formation of Guarnieri's bodies by entirely different staining methods, such as those of Wasielewski

or Ehrlich, or by means of Unna's polychromemethyleneblue-tannin method, or by Mann's biacid mixture, small bodies (0·5–1 μ) will be noticed, usually at one or both poles of the cell, sometimes indenting the nucleus, but, as a rule, lying some distance from it. These gradually increase in size till they reach 3–4 μ in diameter. The general appearance may be gathered from the photograph No. 13 (Plate XXV). It will be noticed that the body always lies in the perinuclear space. On two occasions appearances were found suggesting that the body had just left the nucleus, the latter being distinctly drawn out and destitute of nucleolar matter. (Fig. 12M, Plate XXIV.)

The CHANGES IN THE EPIDERMAL CELLS, apart from those affecting the cytoplasm (Fig. 12, Plate XXIV.) are best studied in sections of tissue removed during the early part of the third day of vaccination and stained according to the Möller or Wasielewski methods. A typical group of cells is seen in Fig 10, Plate XXIII; they appear two to three times larger in diameter than in normal skin, and adhere firmly together by means of the fibrils which bridge the somewhat dilated intercellular lymph channels. The perinuclear cleft, originally small (No. 1), increases considerably in extent (Nos. 4, 5 and 3), till ultimately the walls of the sac almost touch the periphery of the cell. There is thus brought about an accumulation of the fibrils at the periphery of each cell. The cells derived from a common ancestor are united together more firmly by those fibrils which run through them from base to apex. By the end of the third and fourth day these cells, owing to the pressure exerted by the accumulation of lymph, will have become much stretched. The lymph cannot escape either downwards or laterally, and hence, being pent up in the normal intra-cellular channels, exerts pressure on the resisting stratum corneum, owing to which the latter becomes elevated above its normal level. Inasmuch as the only elements which have resisted the disintegration changes, apart from the contents of the nuclear sac, are the fibrils above mentioned, they will act as ties which tend to bind down the stratum corneum. The latter being, however, pushed upwards by the accumulation of lymph, the bundles of fibrils are rendered tense and will compress any cells which may lie between them and thus lead to their atrophy. In this manner the greater number of the giant cells formed in the upper layers of the epidermis eventually become destroyed.

It is difficult to offer a satisfactory explanation of the enormous enlargement of the perinuclear sac which is found to take place. If it arise in the first instance by colliquation, *i.e.*, the flowing together of a number of small vacuoles, this process cannot explain the subsequent increase. On careful focussing, the cytoplasm is seen to be so sharply marked off from the space as to suggest the presence of a special membrane. The theory which at present appears the most feasible is, that by the rarefaction of the cytoplasm, a number of substances are set free which are capable of giving rise to a process of endosmosis, which latter brings about a dilatation of the sac.

In the stratum granulosum the cells increase considerably in their vertical diameter and the granules become very evident when stained by polychrome-methyleneblue (Fig. 15, Plate XXVI.) If it were not for slight differences in the size of

these granules and their occasionally irregular outline, they might readily be mistaken for diplococci, as they frequently are arranged in pairs. The same figure (15, Plate XXVI.) also shows to what an enormous extent the intercellular channels may become dilated, thereby leading to subsequent atrophy of the cells.

Fig. 16, Plate XXVI., is taken from the same preparation but somewhat nearer to the site of inoculation. Many of the cells in the upper layers of the epidermis show a premature conversion into those distinctive of the stratum granulosum. The granules in the latter are derived from the remains of the cytoplasm and not from the cell-fibrils as has been stated.

The cells in immediate contact with the fully keratinised cells of the stratum corneum become considerably distended and, owing to the rich lymph supply, the nuclei, which under normal conditions would only stain feebly or not at all, now stain deeply, owing to the formation of nuclein. This rejuvenescence is, however, but short-lived, as necrosis soon sets in and the cells take part in the formation of the "crust." At the margin of the affected area, many nuclei, as already pointed out, contract into apparently homogeneous spheres which possess a strong affinity for fuchsin.

During the fourth and fifth day of vaccination, necrosis of the lowermost cells in contact with the dermis sets in, and by the sixth day extensive retrogressive changes become noticeable in the cells lying at a somewhat higher level. What is the cause of these necrotic changes will become apparent later on.

To return to the consideration of Guarnieri's bodies. Are all the different structures which have been described under this heading derived from one and the same original element? Have the various forms described by Häckel a common origin? It would appear to be necessary to distinguish a distinct eosinophilous element which is of nucleolar origin (Fig. 12 L and M, Plate XXIV.), and usually placed at the poles of the nucleus, and secondly, a basophilous structure, cytoplasmic in origin, which is usually seen best in those cells in which the nuclei stain deeply in polychrome-methyleneblue-tannin preparations (Fig. 12K, Plate XXIV. and possibly the body marked G.B. in No. 4, Fig. 10, Plate XXIII). There is also a third body which is neither of nucleolar nor cytoplasmic origin, but which consists of matter secreted by the nucleus. It may happen that this secretion is precipitated round the bodies of nucleolar origin, or both may be found separately. Thus the cell No. 2, in Fig. 10, Plate XXIII., had two eosinophilous nucleolar bodies lying in the next serial section, while the large crescentic-shaped body, which is depicted, represents the nuclear secretion.

As the staining reactions of these crescents are exactly the same as that of the cytoplasm (*vide* description of Fig. 12, Plate XXIV.) the suggestion may be hazarded that these demilunes, crescents, or ringlike bodies represent material which under normal conditions would pass directly into the cell-plasm, but which is prevented from so doing owing to the formation of the perinuclear space. The secretion in adapting itself to

the surface of the nucleus produces the peculiar appearances met with.

The degenerative changes which these crescentic masses eventually undergo have been well described by Häckel.

That, very rarely, leucocytes have the power of entering epithelial cells, but only to undergo a peculiar degeneration, is shown by the figure 12R, Plate XXIV., the spherical masses having the same appearance as those already described by M. Heidenhain for salamander leucocytes.

As to the nuclear division induced by the vaccine virus, the cell No. 3, in Fig. 10, Plate XXIII., contains four nuclei, two of which lie in the next serial section; No. 2 possesses two nuclei only, while Nos. 4 and 5, are mononucleated. It is thus evident that the cells close to the stratum corneum may increase considerably in size without undergoing nuclear division. During the fifth and sixth day of vaccination one commonly finds at the margin of the vaccine vesicle giant cells containing as many as a dozen nuclei, which occasionally are arranged in a definitely crescentic form.

If then Guarnieri's bodies do not constitute the organism specific to vaccinia, is it possible to demonstrate anything else that may more probably do so? With all reserve, attention may be drawn to certain appearances found during the second and third day after vaccination in the cell-plasm of the epithelial cells. In Fig. 10, Plate XXIII., 48 hours, and in Fig. 11, Plate XXVI., 120 hours after vaccination, stained by Möller's method for spores and well differentiated, a number of exceedingly small granules, varying from 0·2–0·25μ in diameter, can be seen. These elements are most distinct close to the perinuclear sac, because there the thinness of the cell is more marked. The granules are usually arranged in pairs, they lie between the epithelial fibrils, and are very numerous. Only a few of the most conspicuous granules have been drawn, so as to simplify the figure.

Are these bodies micro-cocci or are they merely a granular precipitate? The question cannot be definitely settled until a dependable method of cultivating the vaccine virus in artificial media outside the animal body has been devised.

Minute Structure of the Dermis.

In the dermis three distinct layers may be distinguished, the basal membrane, the dermis proper, and the hypoderm. The first of these is in direct contact with the epidermis and consists of a very dense feltwork of collagenous fibres giving the characteristic methylblue reaction with Mann's bi-acid mixture.

These fibres fit into the finger-like epithelial processes, and occasionally fine fibrils may be seen to extend upwards within the inter-epithelial lymph spaces, to one-half the length of the lowermost layer of columnar cells.

Underneath this layer is the dermis proper which may conveniently be divided into a superficial vascular and a deep elastic layer. The former consists of collageneous bundles measuring from one-fifth to thirty μ in diameter, between which are the hair follicles, blood vessels and lymphatics. The blood vessels, even

down to the finest subepithelial capillaries possess a very distinct reticular envelope of white fibrous tissue, which stains a deep blue colour in marked contrast to the bright red of the endothelium, fixed connective-tissue cells, sebaceous glands and erector pili muscles.

The deeper strata of the dermis are particularly rich in coarse elastic fibres (*vide* Fig. 21, Plate XXVIII.) which run parallel to the surface, giving off finer twigs which pass more or less vertically upwards to terminate under the basal membrane, occasionally even running for a short distance in the latter.

The dermis, as compared with the hypoderm, is richly supplied with blood vessels and lymphatics, and has in consequence a much more open texture The hypoderm consists of coarse bundles of white fibrous tissue (20–100μ in diameter) which are arranged so as to form thick strands. The arrangement of the connective tissue cells is similar to that in tendon bundles. Between these strands of white fibrous tissue are found a few elastic fibres.

One hour after vaccination (Fig. 19, Plate XXVII.) the epidermis is seen divided, the dermis is laid bare, and at the bottom of the wound is a fibrin clot with red and white corpuscles entangled in it. The blood vessels in the neighbourhood are engorged, and in some cases completely blocked by leucocytes. Between the epidermal edges is seen a clot representing escaped serum which appears to possess a granular structure due to the action of the fixing solution employed. Stained by Gram's method the granules are so resistant to the process of decolorisation and so regular as to closely simulate micro-cocci.

This peculiarity led Kent to speak of large numbers of micro-organisms between the lips of the wound. By a special modification of Gram's method, which he has not as yet published, he states that he has succeeded in staining the specific diplo-bacillus of vaccinia, which is said to be always contained in special cells in the deeper layers of the dermis. I have no doubt that he has been misled by appearances, such as shown in the lower portion of Fig. 24, Plate XXIX., which represents a collection of plasma cells stained by Claudius' modification of Gram's method. By the same process some of the nuclei in the deeper layers of the epidermis (Fig. 24) and dermis (Fig. 24 are very resistant to the decolorisation by Gram, and the gentian violet is apt to be precipitated in forms which resemble bacilli. That these appearances are not pathogenic is shown by their occurrence in perfectly healthy tissues.

Changes in the Dermis due to Vaccination.

The first change to be noticed in the fixed connective tissue elements is a necrosis of the basal membrane, 48 hours after vaccination (*vide* Fig. 9, Plate XXIII.). This swells up and if stained by the short bi-acid method, exhibits a great affinity for

the eosin rather than the methylblue. What is remarkable is that this change should take place beneath the vaccinated area, even at a point distant from that at which the epidermis is divided, and at a time when the changes in the epithelium are as yet but slight.

Commencing with the second day and reaching its height on the fifth day, an œdema of the dermis occurs accompanied by leucocyte infiltration. Primarily in the centre, and later, at the margin of the affected area, some special chemotaxis seems to call forth an emigration of the eosinophilous and neutrophilous leucocytes. Thus we find in Fig. 22, Plate XXVIII., a small blood vessel from which a considerable number of cells have escaped. Before discussing the probable fate of these cells it will be best to shortly summarise some papers dealing with the question.

In 1892, P. Doehle described as to be found in the blood of patients suffering from smallpox, measles, scarlet fever and syphilis, small spheres (0·5–1μ) which were either homogeneous or contained a highly refractile nucleus surrounded by a clear zone. They showed movements, which in some instances could be observed to be due to the action of a flagellum four to five times the length of the body. Occasionally two spheres were enclosed in a common capsule. He also noticed granular and amœboid elements (2·5μ), and ill-defined rodlike protoplasmic bodies possessing small flagella. The structures referred to were found from about the third to the tenth day after the eruption. In the clear lymph removed shortly after the formation of the vesicle he found, in addition to white and a few red corpuscles, bodies resembling those present in the blood. Many of the larger ones showed flagella 0·5–1μ long. During this period he also occasionally observed bodies (2–2·5μ) which constantly changed their shape, being sometimes rounded and sometimes bean-shaped. He expressed the opinion that the smaller elements decrease in number, while the larger ones increase during the later stages of development of the pustule. They, further, never lie included in epithelial cells, during the early stages, but may do so later on.

In 1893 Buttersack described what were apparently the same elements, 50 hours after vaccination, although his view of vaccine fibrils breaking up into globules (sporulation) was due to his method of examining films of lymph dried by exposure to the air.

In 1894 Kanthack and Hardy studied the behaviour of the leucocytes of the frog when brought into contact with anthrax bacilli, and arrived at the conclusion that the eosinophilous cells discharge their granules over the bacilli, and that the work of destruction is completed by the hyaline cells.

In 1896 Weber made further investigation of the blood of patients suffering from variola, and enumerates as occurring therein :—

(1) Spherical highly refractile granules, 1·8μ in diameter, which show Brownian and occasionally progressive movement; (2) granules measuring 5·6–7·2μ, found in the interior of non-mobile,

homogeneous and spherical elements, which he supposed to represent a phase in the life-history of a protozoon, and so termed *Sirenenkörperchen*; (3) large granules occasionally containing smaller ones, a transition towards the formation of the previously mentioned stage; (4) granules joined by threads; (5) stages in the formation of larger bodies from the growth of small granules, and of the division of fully formed bodies; (6) forms measuring 15μ, with one or two opaque and one clear nucleus.

Weber states that his bodies are very like eosinophilous cells, and that they only differ from leucocytes in possessing actively moving granules. He obtained cultures in alkaline semi-solid agar solution, which were invisible macroscopically. As the bodies described by him were also found in measles and scarlatina, it appeared doubtful as to whether they could in any way be regarded as specific.

Walter Reed also found in the blood of monkeys and calves, during the active stages of vaccinia, granular amœboid bodies, having one third the diameter of the red corpuscles. He later discovered them in normal blood, and therefore believed that no causative relationship existed between these bodies and vaccinia.

H. F. Müller, too, discovered, independently of Reed, in normal blood, granules which differed essentially from blood platelets.

In 1897 Stokes and Wegefarth arrived at the conclusion that the bactericidal power of the leucocytes and of the blood serum of man and many animals is due to the presence of specific granules, especially those of the eosinophil and neutrophil type. When called upon to resist the action of invading bacteria the granular leucocytes can give up their granules to the surrounding fluids and tissues.

The rapidity with which, after vaccination, changes occur in the epidermis and dermis varies enormously in different calves. In this respect it may be pointed out that Figs. 2 (Plate XX.) and 22 (Plate XXVIII.) represent the appearance of the dermis in a calf which had been vaccinated 48 hours previously, and which "had not taken well." The corresponding epidermis is shown in Fig. 9 (Plate XXIII.), and it will become apparent that in this region the vaccine lymph was not brought into direct contact with the dermis. Notwithstanding this fact the basal membrane is necrosed beneath the site of inoculation, and, moreover, marked changes extend for nearly 3mm. to either side of this point.

Sections stained in methylblue-eosin mixture show large numbers of eosinophil and neutrophil leucocytes. In most of these (Fig. 23 No. 1, Plate XXIX.), the granules are arranged round one side of the nucleus, some cytoplasm occasionally lying outside the granular zone.

No. 3, in which the nucleus lies to the right, while the cytoplasm with its reticular arrangement is on the left, probably marks the initial step towards a shedding of the granules. Whether No. 4 represents a further step in the transformation of the leucocyte or whether it precedes the condition seen in No. 3 is doubtful, but ultimately the nucleus leaves its excentric position and becomes central, a change accompanied by a rarefaction of the nuclear contents (No. 5). These leucocytes cannot be seen to multiply by either direct or indirect division. It may be well to state that precautions were observed to exclude the possibility of appearances such as those seen in Nos. 3 and 4 being due merely to the nonstaining of basophil granules.

In Fig. 23, Plate XXIX. in addition to the leucocytes, numerous granules are seen in the lymph spaces between the collageneous bundles. These bodies vary in diameter from 0·75 to 1·5μ; a few are spherical but the greater number are irregular in outline. They are not improbably identical with those which the various authors above-mentioned have found in the blood and also in the lymph of vaccinia and variola during the febrile stages and the further suggestion may be hazarded that they are in reality leucocyte granules which have been discharged from the cells. As it is, however, as yet premature to make dogmatic assertion on this point, these elements may for the present be conveniently termed "Z granules."

Whatever the origin of these Z granules may be, they seem to possess the powers of amœboid movement and growth, as Figs. 18 (Plate XXVI.), 25, 26 (Plate XXX.), and 28 (Plate XXXI.) appear to show. These figures represent the appearances observed 72 hours after vaccination. The photograph (Fig. 28) shows how numerous these elements may be, while Fig. 25 demonstrates the great differences in their diameter; the larger granules measuring fully 3μ. The preparation here figured was treated as follows: (1) Ten minutes in 1 per cent. acetic acid, (2) three days at 30° C. in Löffler's methyleneblue containing ten times the normal amount of KOH, (3) 2½ hours in 1 per cent. acetic acid, (4) absolute alcohol and xylol. The section has a uniform bluish-black tint, quite different from that ordinarily obtained with methyleneblue.

That the granules are amœboid seems probable because of the various shapes which they exhibit (Fig. 18, Plate XXVI.), because they may be found at considerable distances away from leucocytes, and because they are met with also in the narrow septa which separate the bundles of white fibrous tissue (Fig. 26, Plate XXX.)

Sections stained for 10 minutes in undiluted polychrome-methyleneblue, washed for 20 seconds in distilled water, and subsequently differentiated in a 33 per cent. solution of tannin (Unna) for two to five minutes, show the appearances depicted in Fig. 26. The Z granules, with high focus, stand out as highly refractile elements, either not stained at all or of a faint green colour. In mid-focus they exhibit a minute spherical or elongated granule of a deep blue colour. Whether this appearance can be

explained on purely physical grounds as being only due to a partial decolourisation of the Z granules is not certain, for in many instances the central globule will appear of the same size although the Z granules, as a whole, differ greatly in their diameter.

Unna's staining method is specially useful for tracing the changes in the fixed and wandering connective tissue cells, both nuclei and the cell bodies with their winglike processes being well stained.

By it the leucocyte, Fig. 15 (Plate XXIV.), was discovered stretching throughout the thickness of the epidermis, and measuring 126·7μ in length.

By yet another method, namely, by staining for 12 hours in Löffler's methyleneblue solution containing 1 : 1000 KOH, then differentiating in 2 per cent. H_2SO_4 it is possible to demonstrate in sections of skin, removed 72 hours after vaccination, cells apparently identical with various forms of Waldeyer's plasma cells. These are represented on Plate XXIV., Fig. 14 a–f. In the sections they appear as cherry-red elements, the remainder of the section being stained a bright blue. What is characteristic of the plasma cells is the presence in them of granules which are smaller than those of the eosinophilous leucocytes. The cells may appear rounded or provided with processes, their average length is 14–15μ and their nucleus under normal circumstances measures about 5 × 7·5μ. Therefore they are much smaller than the winged connective-tissue cells, some of which (Fig. 25, Plate XXX.) extend with their processes over 50μ.

The granules of these plasma cells stain of a bright cherry-red colour, as long as the nucleus stains blue, but in most cells the nucleus has lost its blue tint and is apparently undergoing degenerative changes. The healthy appearance of the cell is seen in Fig. 14a, Plate XXIV., in which the nucleus has distinct chromosomes, and the perinuclear area contains but few granules which, however, stain deeply; in Fig. 14b, Plate XXIV., the nucleus still stains with methyleneblue, but has a vacuolated appearance, and the granules are less deeply stained than in a; in b, d, e and f the nucleus stains with methylene violet of the same tint as the granules (some of which are feebly stained or quite invisible) and has a more or less vacuolated appearance.

The reason for mentioning the plasma cells here is to point out a possible relationship between the appearances obtained by the method above detailed and that seen in methyl-blue-eosin sections (Figs. 22 and 23, Plates XXVIII. and XXIX). In the former figure a number of cells are surrounded by eosinophilous granules, which are somewhat smaller than those of leucocytes, and which show a greater affinity for eosin. They correspond to the granules figured in the upper fourth of Fig. 23, Plate XXIX. Can it be that plasma cells set free their granules?

APPENDIX C

On the Histology of Vaccinia; by Drs. Copeman and Mann.

Commencing with the second day of vaccination and reaching its height at about 120 hours, the œdema of the dermis leads to the marked changes represented in Figs. A, B and C, Plate XXXIII. These three photographs are taken from the same section (120 hours after vaccination). Fig. A, Plate XXXIII., is farthest away from, and Fig. C, Plate XXXIII., nearest to the point of inoculation. The section was stained in a mixture of gentian violet one part, glacial acetic acid two parts, alcohol five parts, made up to 100 parts with water. The epidermis stained red while the collagenous bundles stained a deep blue. This method is specially adapted for studying the relationship of the dermis to the epidermal cells, the connective tissue processes springing from the basal layer, standing out sharply. In Fig. A., Plate XXXIII, the normal connective tissue appears very darkly stained as the section was 10μ thick, but nevertheless indications of lymphatics and blood vessels can be seen. The epidermal cells are normal in appearance. In Fig. B, Plate XXXIII., the connective tissue elements have been pushed asunder by the accumulation of lymph, the basal membrane being, however, as yet not perforated. The epidermal cells are markedly enlarged and stain less readily. In Fig. C, Plate XXXIII., the collagenous bundles appear as very delicate trabeculæ, the basal membrane is giving way in various places, and the epidermal elements have been completely destroyed.

In comparing the three figures with one another it is well to bear in mind that the delicate reticular arrangement seen in Fig. C, Plate XXXIII., is not due to an actual diminution in the total amount of white fibrous tissue present, but owing to the œdema the tissue elements have become spread out over a larger area. Actual destruction of the connective tissue is not one of the primary effects of the vaccine virus, and when it eventually occurs is due partly to the direct action of extraneous micro-organisms which first become noticeable about the third day, and partly to the simultaneous accumulation of leucocytes which, by the pressure they exert tend to bring about atrophy of the white fibrous tissue.

As Unna points out, during the vesicular stage of vaccinia the inflammatory changes are small and leucocyte infiltration is at first remarkably scanty.

To demonstrate the presence of extraneous micro-organisms subsequent to the third or fourth day, either the method detailed above for Z-granules, by the use of the modified Löffler stain may be adopted, leaving the sections at 30° C. or the sections may be stained for 12 hours at 45° C. without previous treatment with acetic acid, the stain being rapidly washed off before the slide has had time to cool and the section then cleared in xylol and mounted in balsam. By this second method the appearance shown in Fig. 17, Plate XXXVI., was obtained 96 hours after vaccination. Diplococci 0·75–1 μ

in diameter are seen surrounded by a large number of leucocytes. To the left of the preparation is an epithelial cell in an early stage of necrosis.

Fig 27, Plate XXXI., stained by the acetic acid modified Löffler method was taken from a section 72 hours after vaccination. In it big bullae are seen which have resulted from a separation of the lowermost epithelial cells A from the middle layer B. Free in the lymph minute diplo-bacilli will be observed.

In the description of the reticular change in epidermal cells given previously, certain granules, best seen in Fig. 10, Plate XXIII., were described which resemble diplococci. By using the same staining method, namely that of Möller, a similar appearance can be demonstrated during the second, third and fourth day in the œdematous connective tissue, as may be seen in Fig. 18, Plate XXXVI, taken from a section 72 hours after vaccination. Numerous small granules, single or arranged in pairs, are found between the bundles of connective tissue.

How minute these granules are may be gathered by comparing them with such undoubtedly extraneous micro-organisms as are seen in Fig. 17, Plate XXVI.

In conclusion reference must be made to the Figs. 29–31, Plate XXX., which show that up to the 72nd hour after vaccination leucocyte infiltration is limited to the dermis proper, because the extraneous organisms which are the exciting cause lie in the coagulum filling the mouth of the wound and also because the dermis more readily permits of such emigration than does the hypoderm. In the latter but few blood vessels are found, as its structure is virtually that of tendon. Fig. 30, Plate XXXII., shows that for the first few days after vaccination the elastic fibres are quite unaffected and this is so till about the 96th or 120th hour, when some of the fibres lying close to the line of incision can be seen to break up into rows of granules, subsequent to which they fail to give the typical staining reactions with Tänzer-Unna's orcein, but still stain with Weigert's resorcin, fuchsin, and ferric-chloride mixture.

Resumé :—

Chronological order of events :—

1 hour after vaccination	: Effects of injuries due to inoculation, viz. : Vasodilation ; emigration of a few leucocytes from vessels directly injured. Stasis of leucocytes in blood and lymph vessels close to site of inoculation.
24–48 hours after	: Marked emigration of eosinophilous leucocytes. Shedding of the Z-granules. Necrotic swelling of the basal membrane.

APPENDIX C.

On the Histology of Vaccinia; by Drs. Copeman and Mann.

48–72 hours after : Swelling of the epithelial structures and hence bulging of the epidermis into the dermis. Accumulation of lymph in the epidermis, causing a breaking up of its cells into strands. Formation of bullae. Some cells atrophy while others hypertrophy. Proliferation of nuclei, especially in the deeper layers. Various elements differing in their origin, but generally described as Guarnieri's bodies, make their appearance. These are not parasites. The Z-granules show amœboid movement and growth. Both in the epidermal cells and in the lymph spaces of the dermis very minute cocci-like bodies are found in large numbers, and in the dermis the Z-granules are found close to these small bodies.

The dermis shows localised swelling. Leucocytes with several nuclei are rare.

72–120 hours after : Invasion of inoculated area by micro-organisms. Few eosinophilous, large numbers of hyaline leucocytes wandering towards the place of inoculation, but as yet they are restricted to the dermis proper. The epithelial "balloons" reach their height of development. Many encapsuled cells resembling the structures described by some authors as cancer parasites are present.

120–144 hours after : Marked necrotic changes in the epidermis and dermis. Invasion of the hypoderm by leucocytes. Necrosis of the leucocytes in the dermis.

PLATE XX.

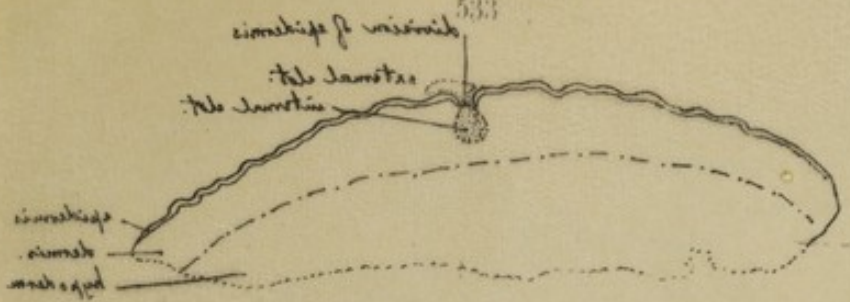
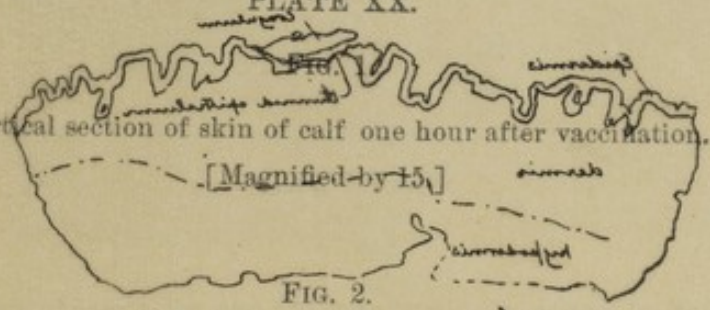


PLATE XX.



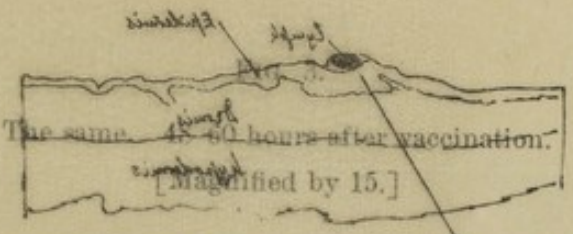
Vertical section of skin of calf one hour after vaccination.

[Magnified by 15.]

FIG. 2.

The same. 48 hours after vaccination.

[Magnified by 15.]



The same. 48 hours after vaccination.

[Magnified by 15.]

FIG. 4.—Same as Fig. 3.

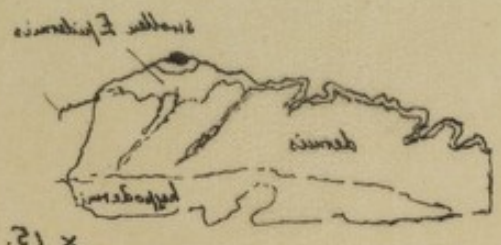


PLATE XX.

FIG. 1.

48 hours after vaccination.

FIG 2.

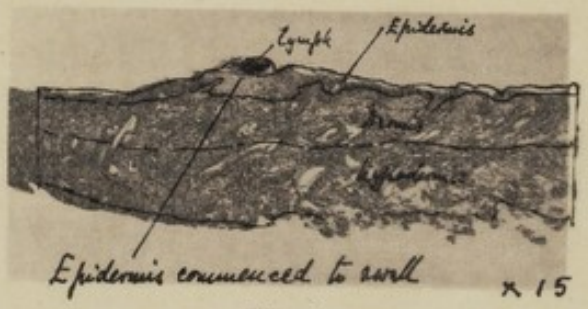


FIG. 3.

FIG. 4.

PLATE XX.

FIG. 1.

Vertical section of skin of calf one hour after vaccination.
[Magnified by 15.]

FIG. 2.

The same. 48 hours after vaccination.
[Magnified by 15.]

FIG. 3.

The same. 48–60 hours after vaccination.
[Magnified by 15.]

FIG. 4.—Same as Fig. 3.

PLATE XXI.

FIG. 5.

Vertical section of skin of calf 72 hours after vaccination.
[Magnified by 15.]

FIG. 6.

The same. 96 hours after vaccination.

PLATE XXI.

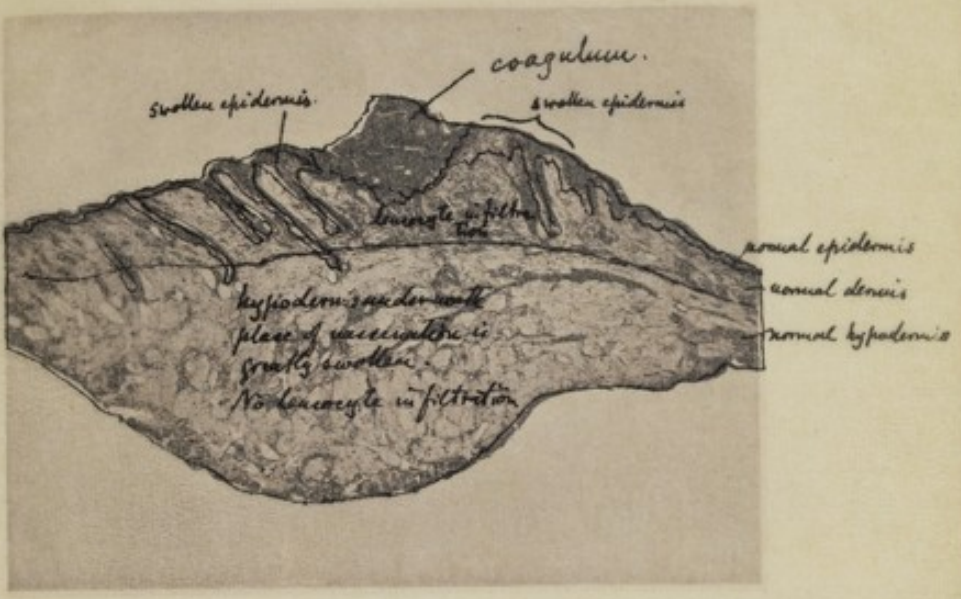


FIG. 5.

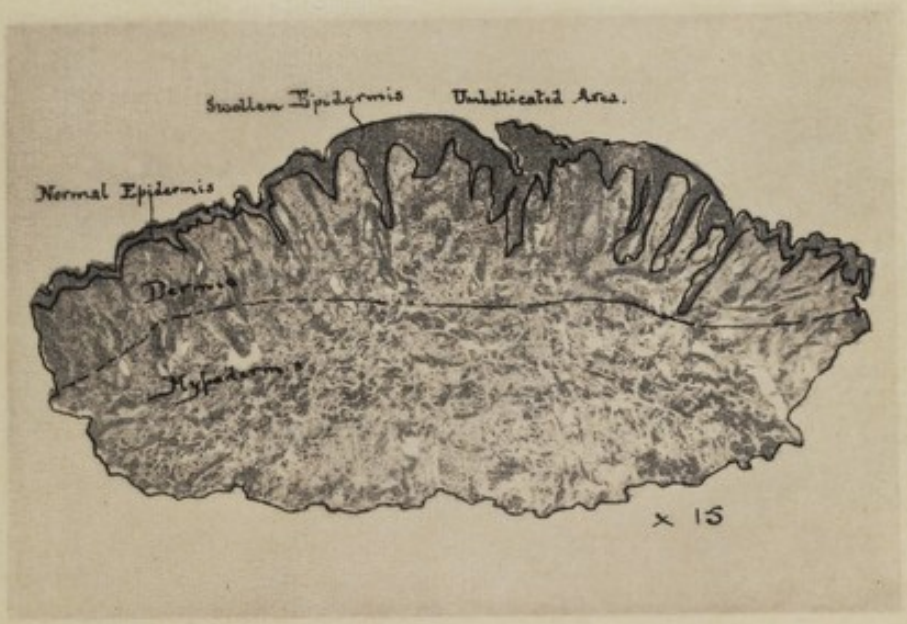


FIG. 6.

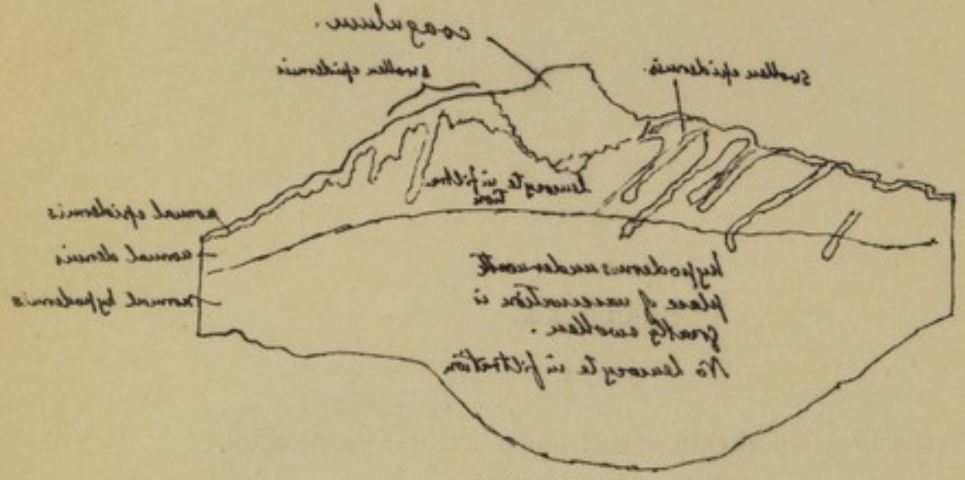


PLATE XXI.

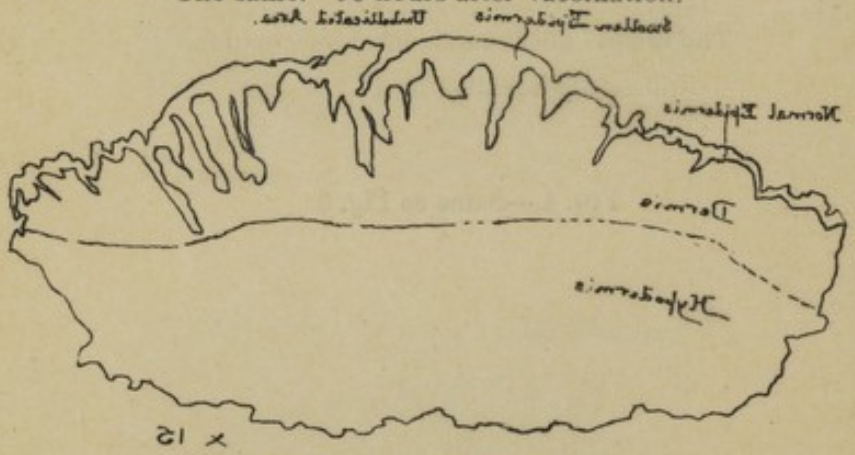
FIG. 5.

Vertical section of skin of calf 72 hours after vaccination.

[Magnified by 15.]

FIG. 6.

The same. 96 hours after vaccination.



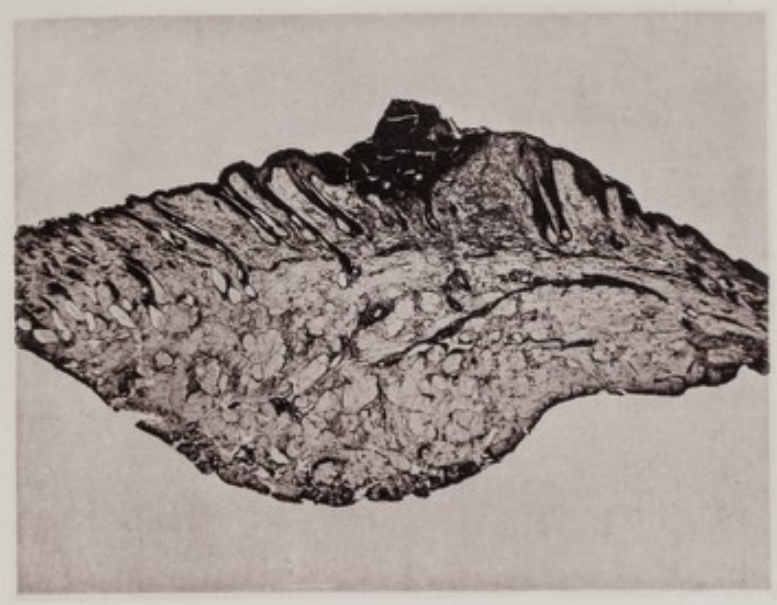


PLATE XXII.

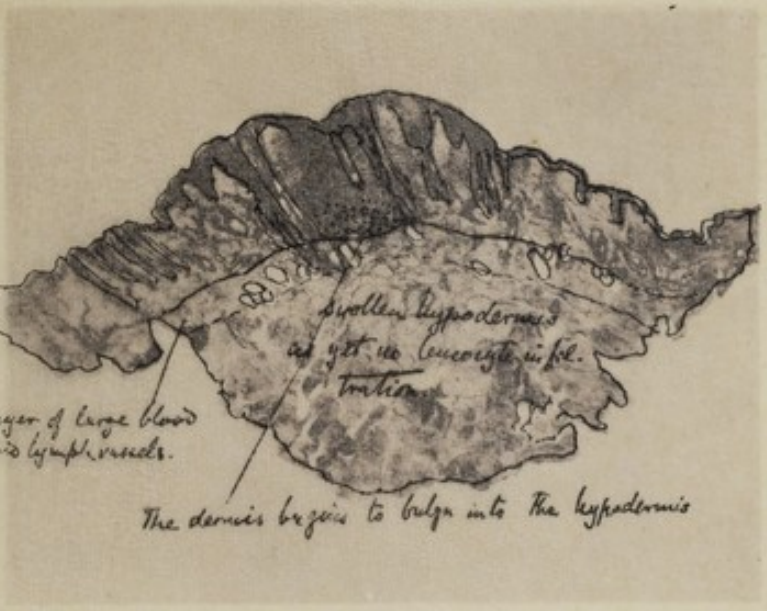


FIG. 7.

FIG. 8.

PLATE XXII.

FIG. 7.

FIG. 8.

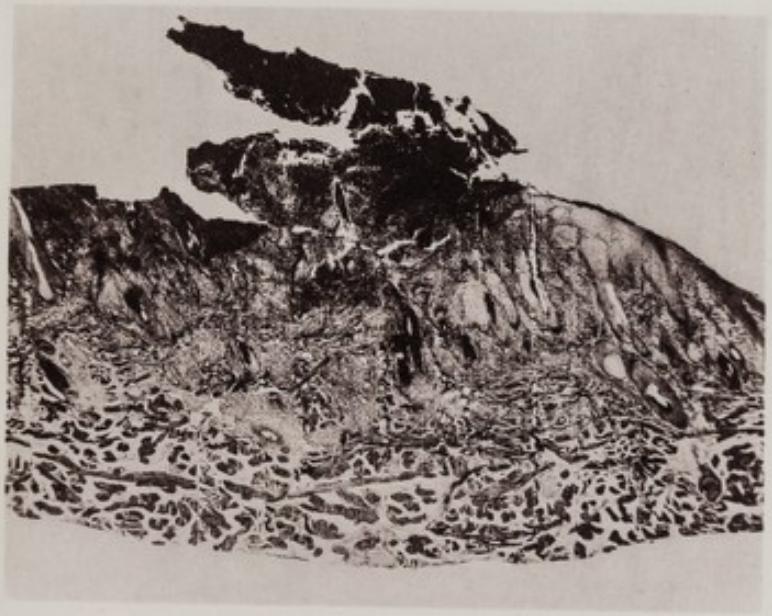


PLATE XXII.

FIG. 7.

Vertical section of skin of calf 120 hours after vaccination.

FIG. 8.

The same. 144 hours after vaccination.

PLATE XXIII.

FIG. 9

Vertical section of skin of calf 48 hours after vaccination.

Epidermal cells with fibrils. Intercellular lymph channels increased in diameter. Necrosis of basal membrane.

FIG. 10.

The same. 72 hours after vaccination. Unna's "reticulating colloquation."

FIG. 11.

The same. 120 hours after vaccination. Unna's "ballooning colloquation."

PLATE XXIII.

FIG. 11.

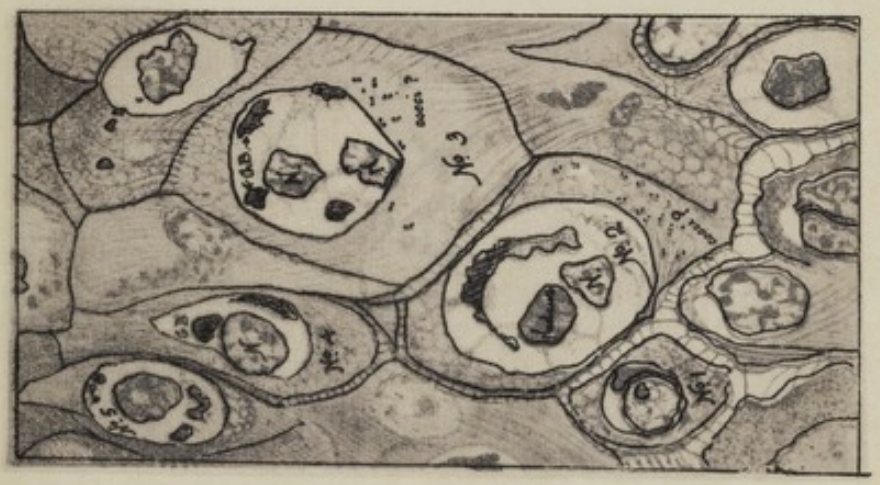


FIG. 10.

FIG. 9.

PLATE XXIII.

FIG. 9

Vertical section of skin of calf 48 hours after vaccination.

Epidermal cells with fibrils. Intercellular lymph channels [illegible] in diameter. Necrosis of basal membrane.

FIG. 10.

The same, 72 hours after vaccination. Unna's "reticulating colloquation."

FIG. 11.

The same, 120 hours after vaccination. Unna's "clavelling colloquation."

FIG. 8.

Fig. 12. A-E. Cytoplasmic changes.

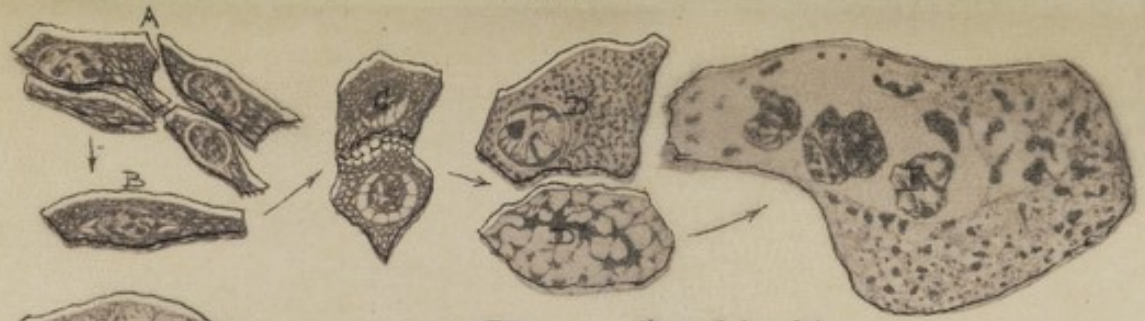


Fig 12. N.-R
Supposed parasites, in reality partly epidermal cells, partly leucocytes.

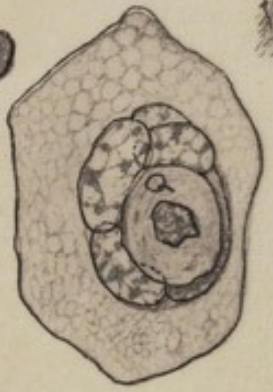
Fig 12
F-I
Cells degenerated most on that side nearest plane of inoculation, while Guarnieri's body is on the distal side of plane of inoculation.

Fig 12
K-M.

10 μ.

Leucocyte measuring 128·7 μ.

Fig 14. a-f
Plasma Cells.



degenerating leucocyte

PLATE XXIV.

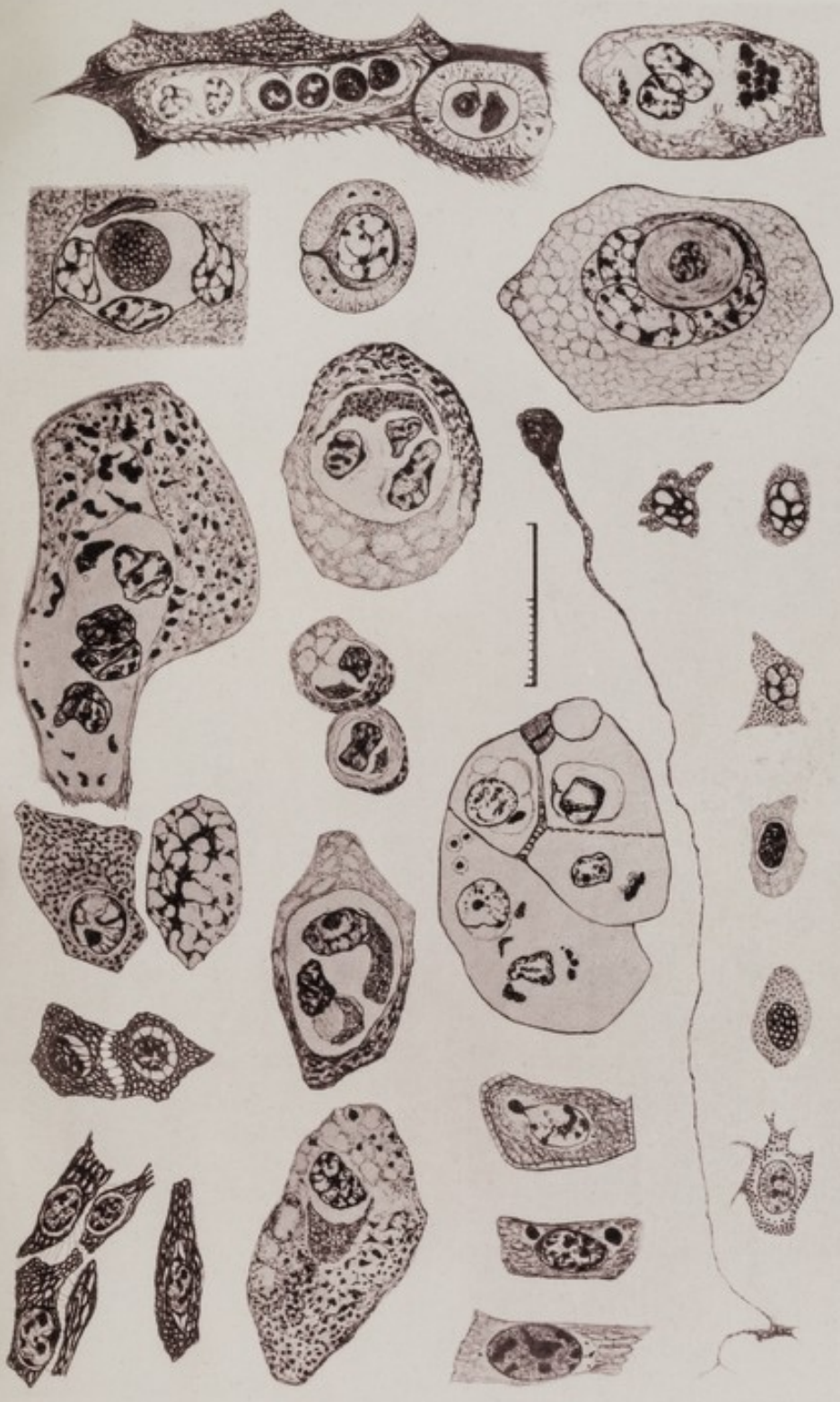


PLATE XXIV.

FIG. 12, A—E.

Changes in the cytoplasm of epidermal cells 72 hours after vaccination.

FIG 12, F—I.

Nuclear secretions forming the crascentic variety of Guarnieri's bodies.

FIG. 12, K.

A basophil Guarnieri's body of cytoplasmic origin.

FIG. 12, L & M.

Nucleolar origin of Guarnieri's bodies

Fig. 12, N—Q.

Epithelial cells simulating parasites.

FIG. 12, R.

Degenerating leucocyte in epidermal cell.

FIG. 13.

Nest of daughter cells derived from a single giant cell.

FIG. 14, *a—f*.

Plasma cells.

FIG. 15.

Leucocyte 126·7 μ in length.

All figures magnified by 1,000, except Fig. 13, which is only magnified by 700.

PLATE XXV.

FIG. 13.

Epidermis, 72 hours after vaccination, showing Guarnieri's bodies.

[Magnified by 300.]

FIG. 14.

Epidermis, 72 hours after vaccination: Formation of bullæ, epithelial strands, and "balloons."

[Magnified by 100.

PLATE XXV.

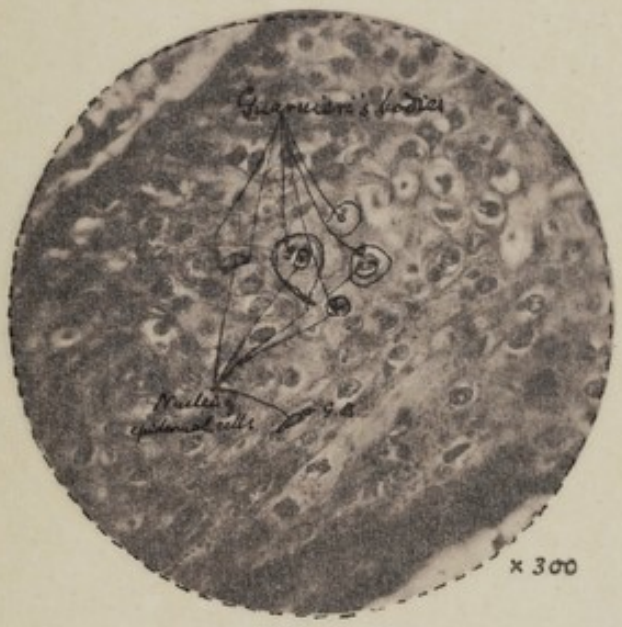


FIG. 13.

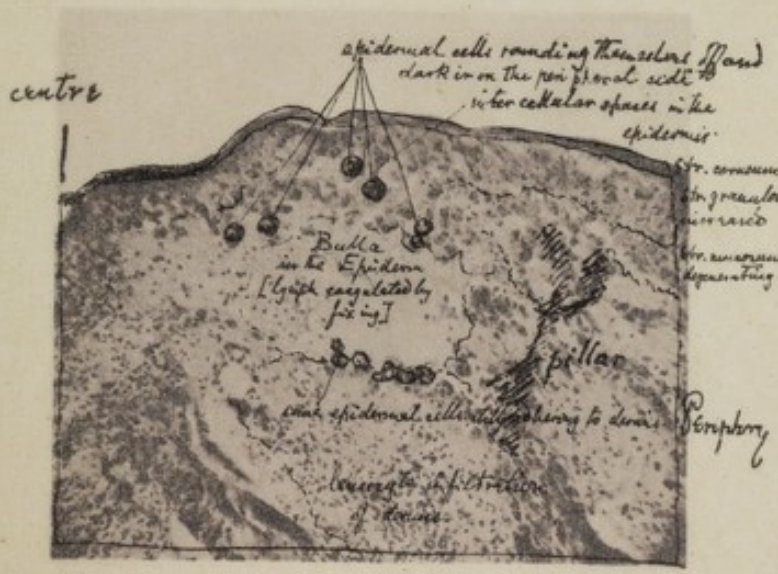


FIG. 14.

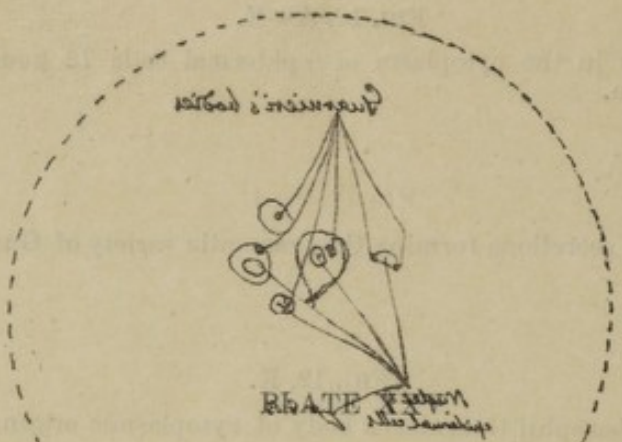


FIG. 13.

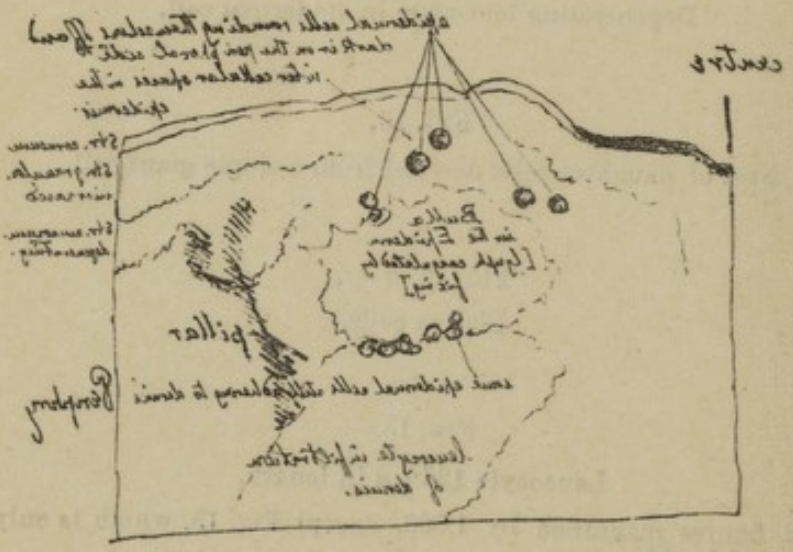
Epidermis, 72 hours after vaccination, showing Guarnieri's bodies.

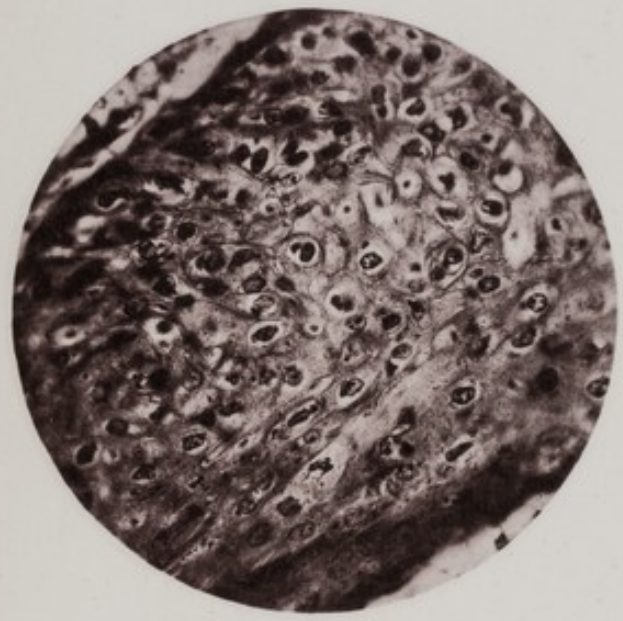
[Magnified by 300.]

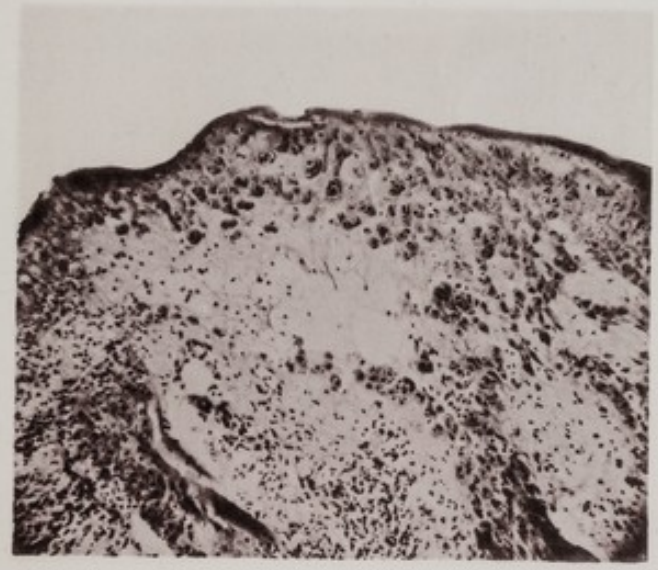
FIG. 14.

Epidermis, 72 hours after vaccination: Formation of bullæ, epithelial strands, and "balloons."

[Magnified by 100.







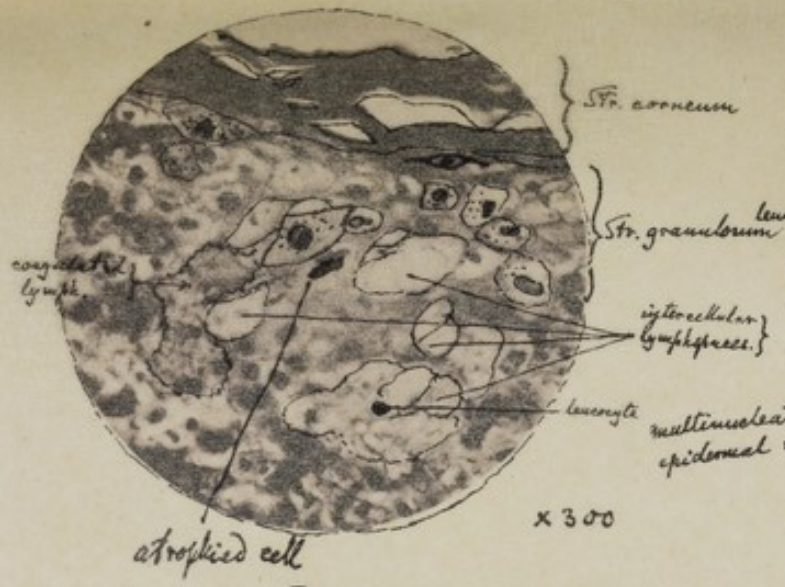


FIG. 15.

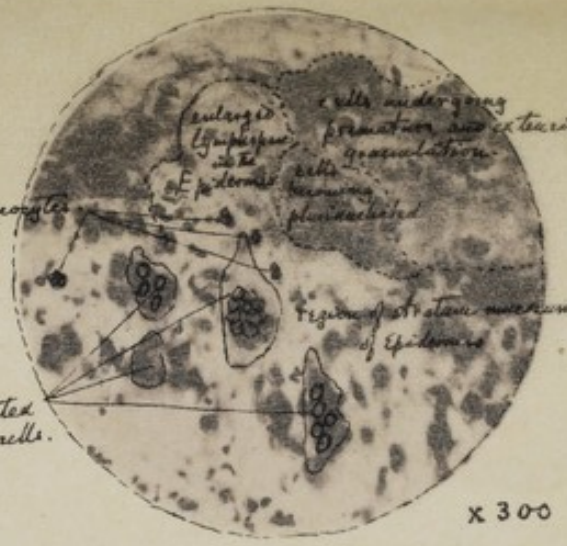


FIG. 16.

FIG. 17.

FIG. 18.

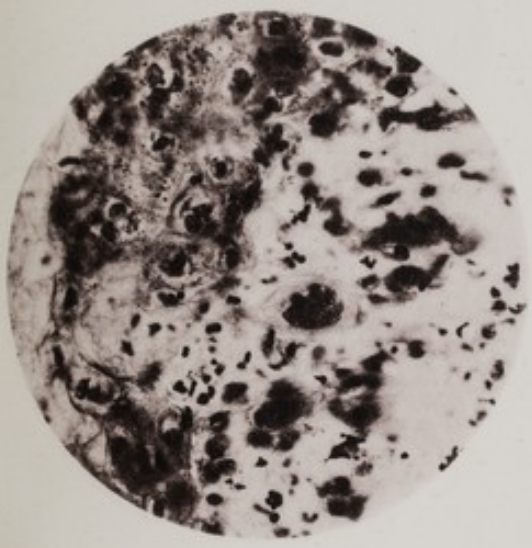
PLATE XXXI.

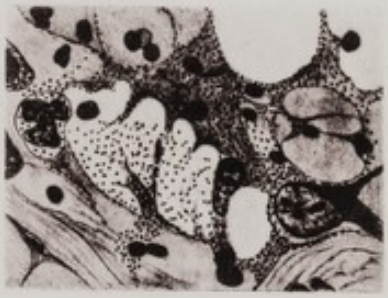
Fig. 11.

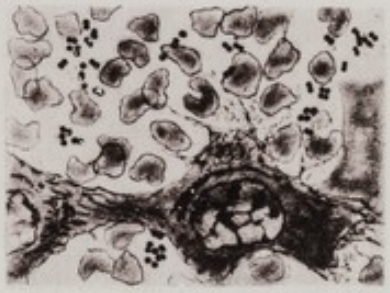
Fig. 12.

Fig. 13.

Fig. 14.







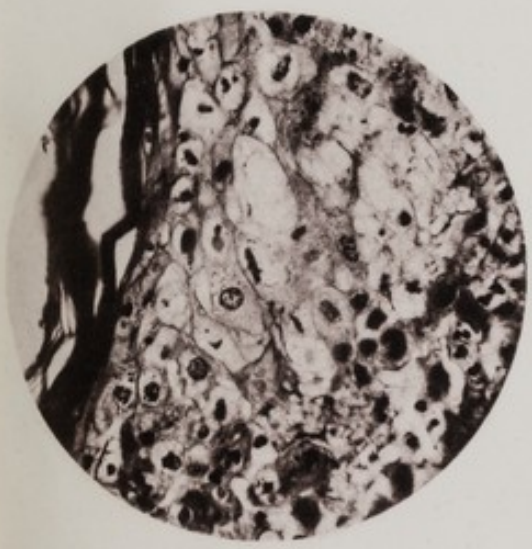


PLATE XXVI.

Figs. 15 & 16.

Upper part of *stratum mucosum* in the epidermis, 72 hours after vaccination. Fig. 15 is nearer the site of inoculation, and in both figures the right-hand portion is that furthest from the plane of inoculation.

[Magnified by 300.]

Fig. 17.

Micro-organisms surrounded by leucocytes 96 hours after vaccination.

Fig. 18.

Minute granules surrounded by the Z-granules, 72 hours after vaccination.

PLATE XXVII.

FIG. 19.

Vertical section of skin of calf one hour after vaccination.
[Magnified by 50.]

FIG 20.

The same. 48 hours after vaccination.
[Magnified by 50.]

PLATE XXVII.

FIG. 20.

FIG. 19.

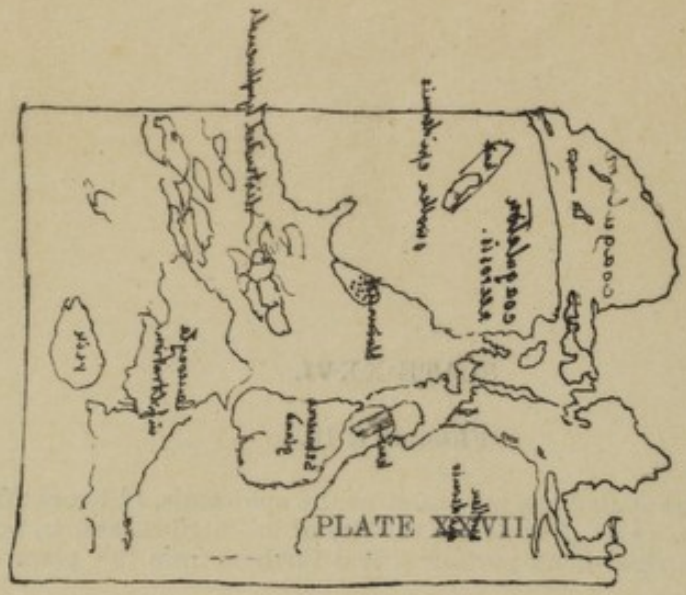


FIG. 19.

Vertical section of skin of calf one hour after vaccination.
[Magnified by 50.]

FIG 20.

The same. 48 hours after vaccination.
[Magnified by 50.]

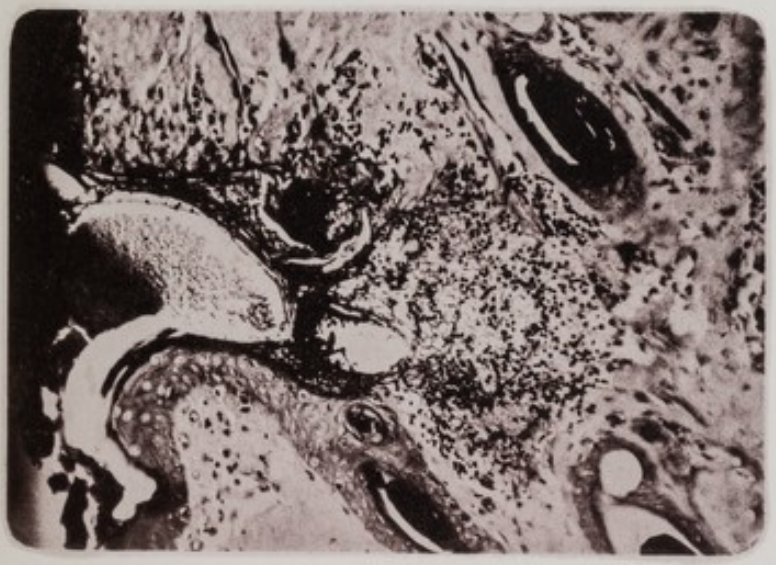


PLATE XXVIII.

FIG. 21.

FIG. 22.

Fig. 21.

Fig. 22.

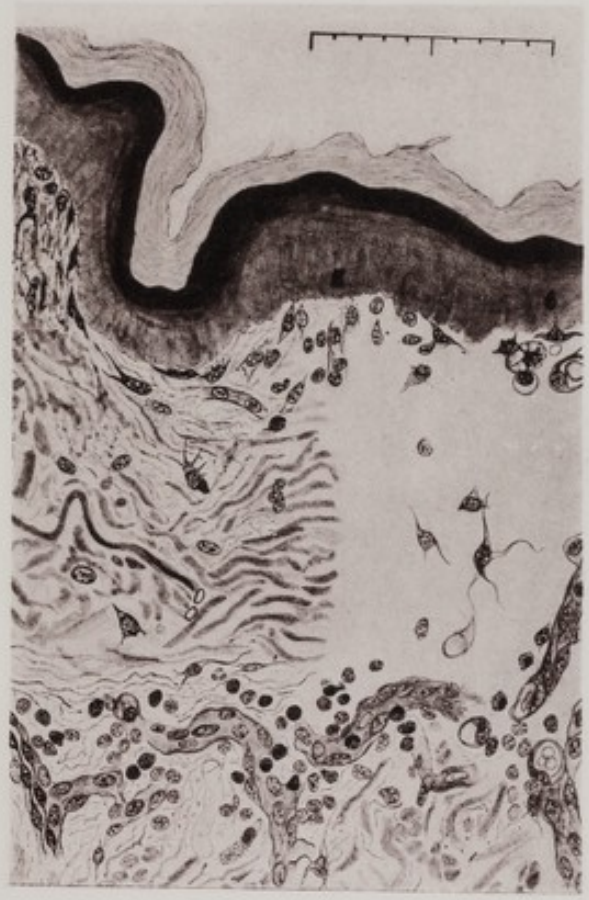


PLATE XXVIII.

FIG. 21.

Periphery of skin-section 72 hours after vaccination. Commencing leucocyte infiltration. The distribution of the elastic fibres is also shown.

FIG. 22.

Periphery of skin-section 48 hours after vaccination.

PLATE XXIX.

FIG. 23.

Z-granules.

[Magnified by 1,000.]

FIG. 24.

Pseudo-bacilli and cocci produced in the eosinophilous epidermal cells, and in plasma cells by Gram's method of staining.

[Magnified by 1,000.]

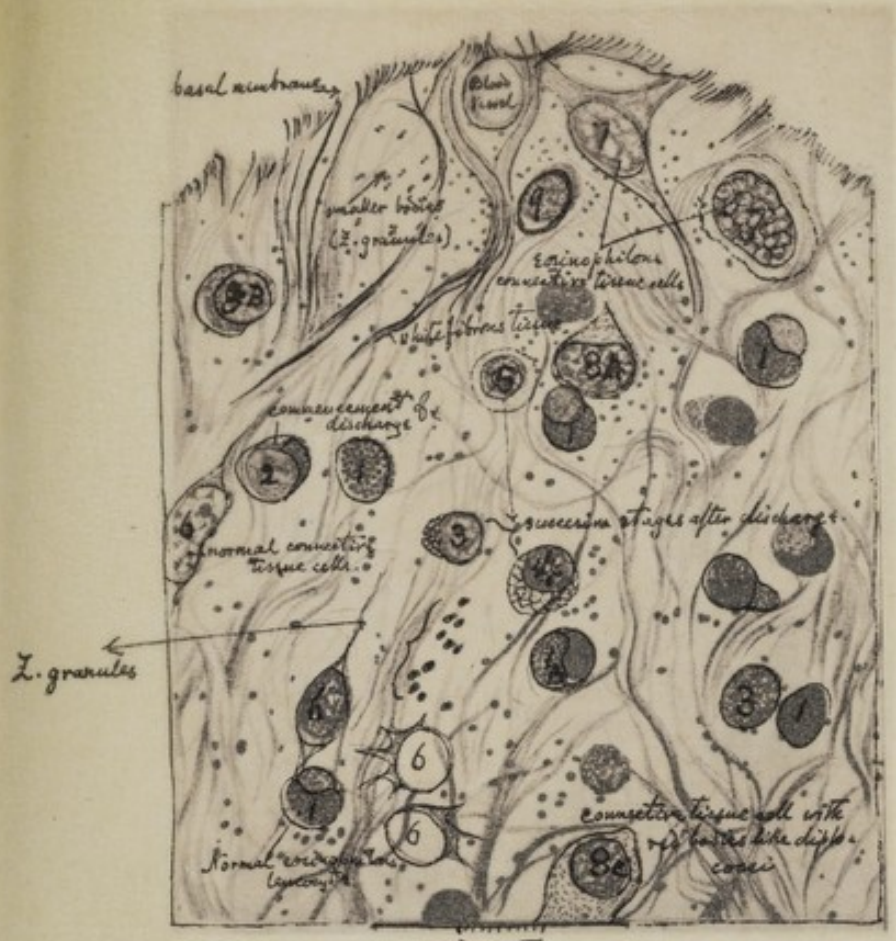

basal membrane
blood vessel
smaller bodies (Z. granules)
eosinophilous connective tissue cells
commencement of discharge
normal connective tissue cells
successive stages after discharge
Z. granules
connective tissue cell with
10μ.

FIG. 23.

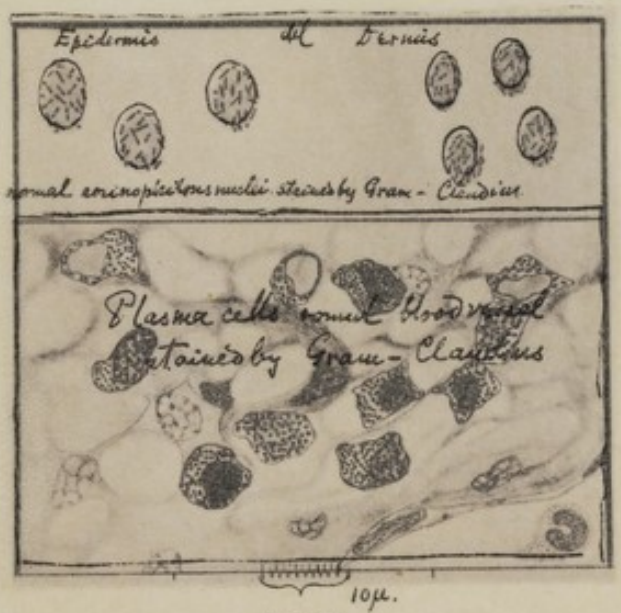

Epidermis
Dermis
normal eosinophilous nuclei stained by Gram - Claudius
Plasma cells normal blood
stained by Gram - Claudius
10μ.

FIG. 24.

PLATE XXIX.

FIG. 23.

Z-granules.

[Magnified by 1,000.]

FIG. 24.

Pseudo-bacilli and cocci produced in the eosinophilous epidermal cells, and in plasma cells by Gram's method of staining.

[Magnified by 1,000.]

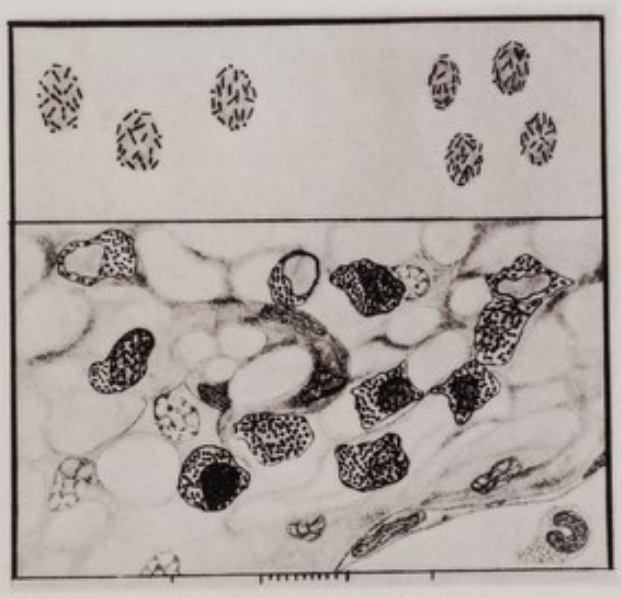


PLATE XXX.

FIG. 25.

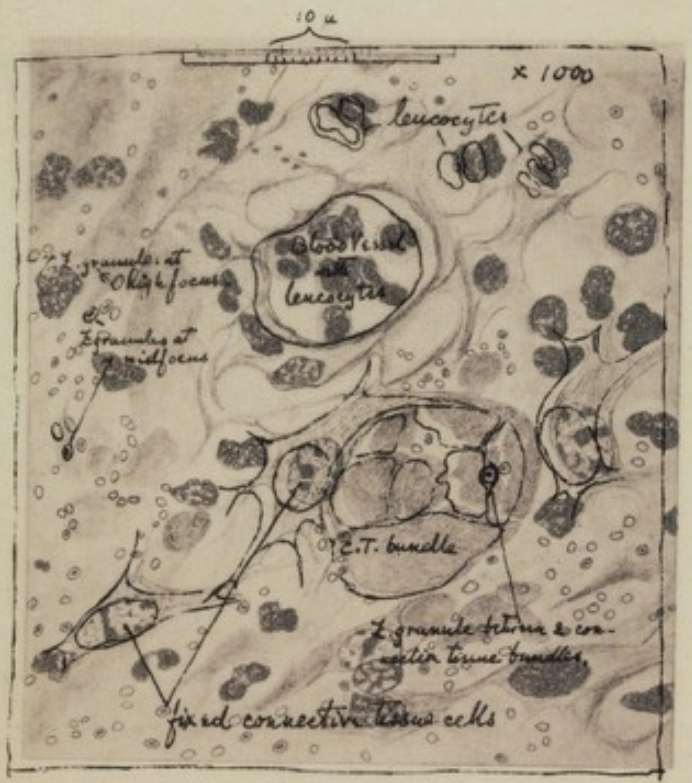


FIG. 26.

PLATE XXX.

FIG. 25.

FIG. 26.

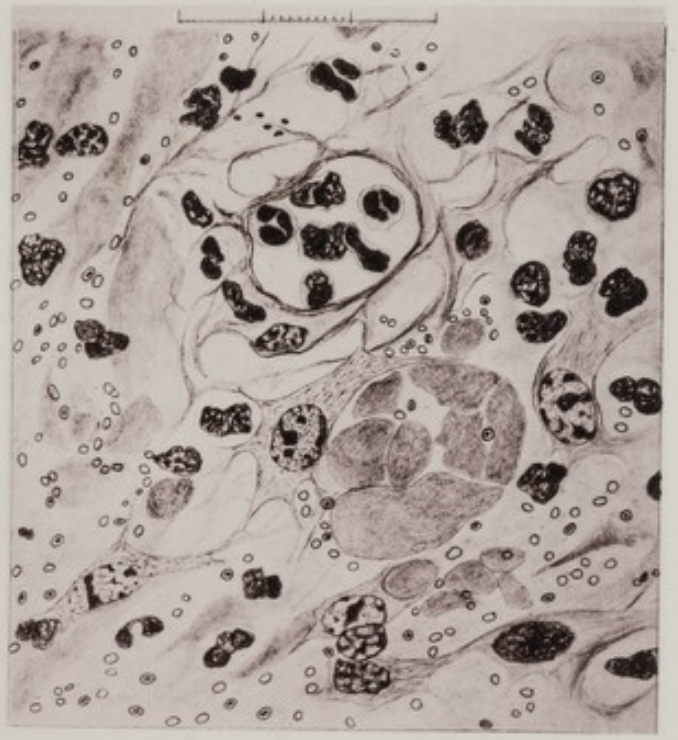


PLATE XXX.

FIG. 25.

Section, stained by Löffler's modified method, showing increase in size of Z-granules 72 hours after vaccination.

FIG. 26.

Similar section stained by Unna's polychrome methylene-blue tannin method.

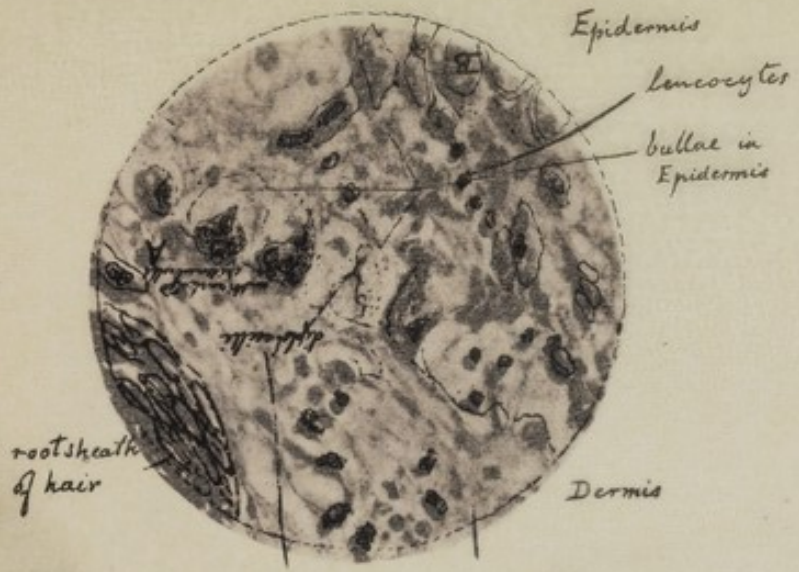
PLATE XXXI.

FIG. 27.

Junction of epidermis and dermis 72 hours after vaccination.
[Magnified by 400.]

FIG. 28.

Microphotograph of Z-granules 72 hours after vaccination.
[Magnified by 500.]



C.T. papilla with greatly enlarged lymphspaces

FIG. 27.

FIG. 28.

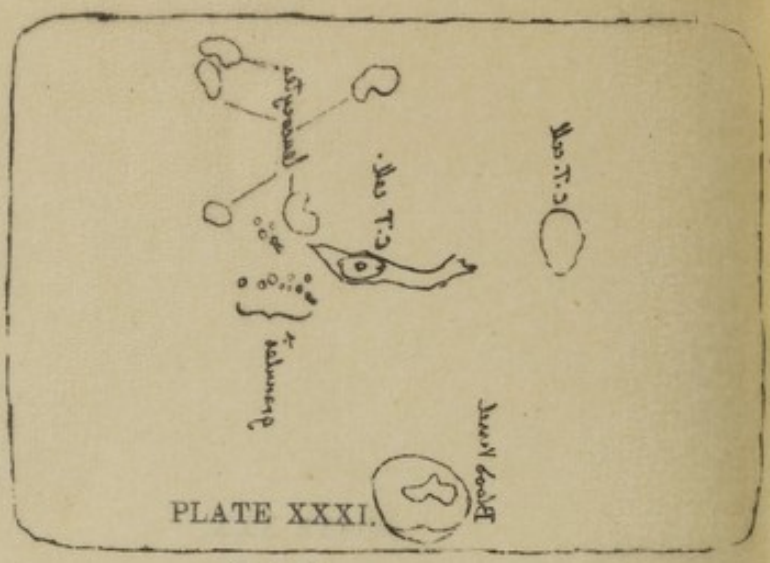


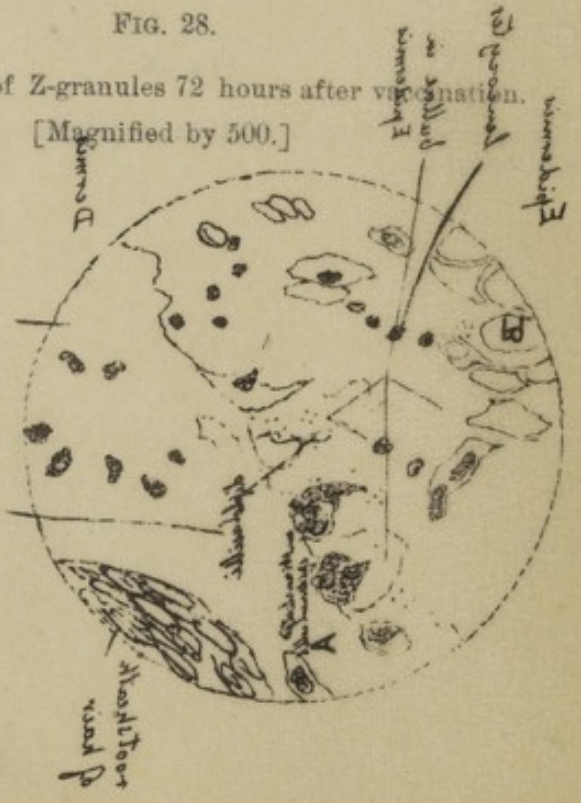
PLATE XXXI.

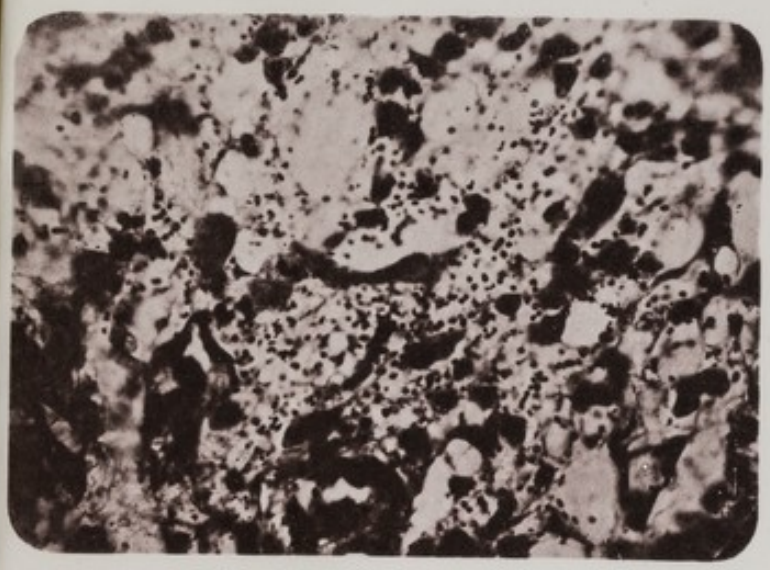
FIG. 27.

Junction of epidermis and dermis 72 hours after vaccination.
[Magnified by 400.]

FIG. 28.

Microphotograph of Z-granules 72 hours after vaccination.
[Magnified by 500.]





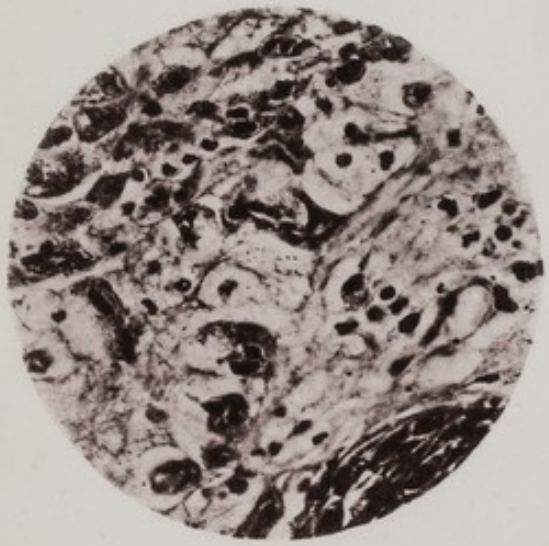


PLATE XXXII.

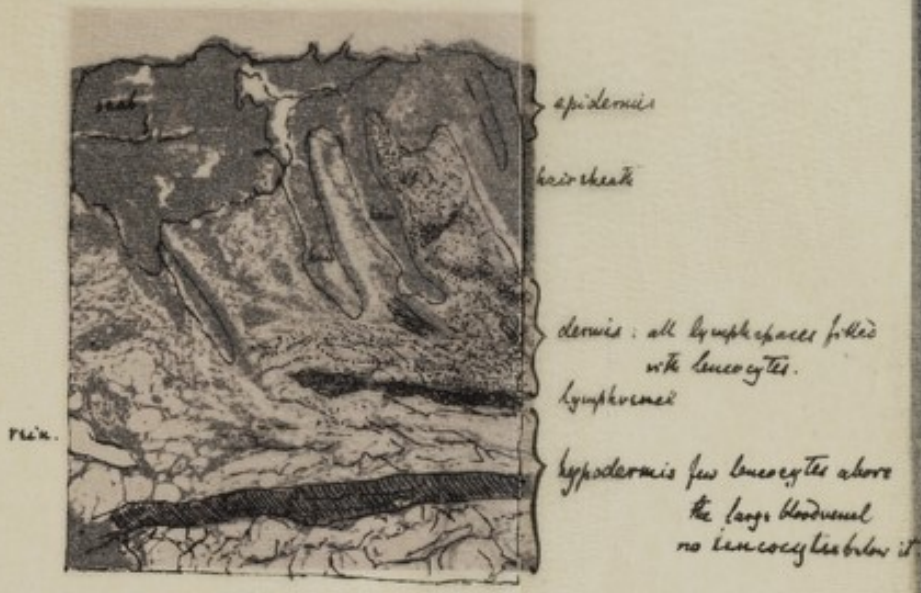


FIG. 29.

...mous leucocyte infiltration of the dermis while the hypodermal elements are not invaded although much swollen

FIG. 30.

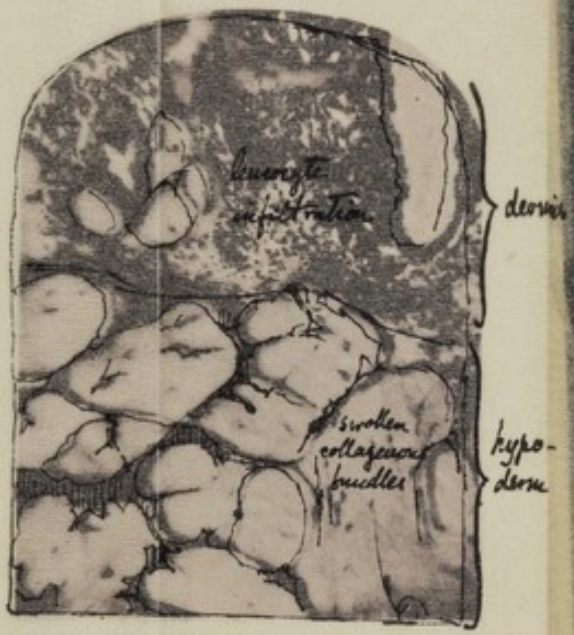


FIG. 31.

Fig. 29.

Fig. 31.

Fig. 30.

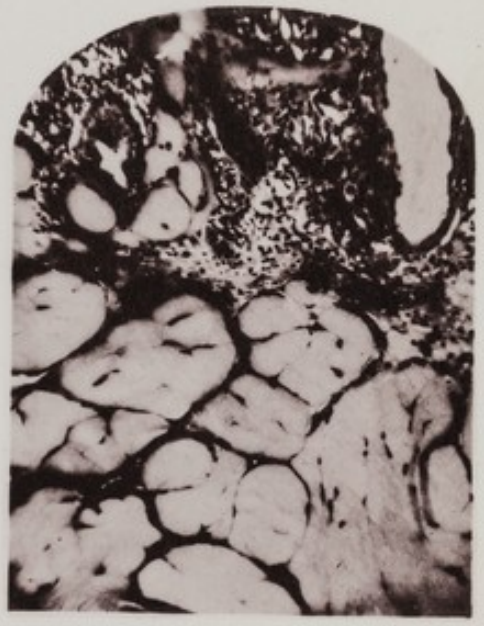


PLATE XXXII.

Fig. 29–31.

Leucocyte infiltration of the dermis 72 hours after vaccination.

Fig. 30 shows the elastic fibres as yet unaffected.
Figs. 29 and 30 are magnified by 50.
Fig. 31 is magnified by 300.

PLATE XXXIII.

Figs. A.–C.

Vertical section of skin of calf 120 hours after vaccination All three photographs are taken from the same section. Fig. A. is taken at the periphery of the section, and Fig. C. close to the line of inoculation.

[Magnified by 300.]

PLATE XXXIII.

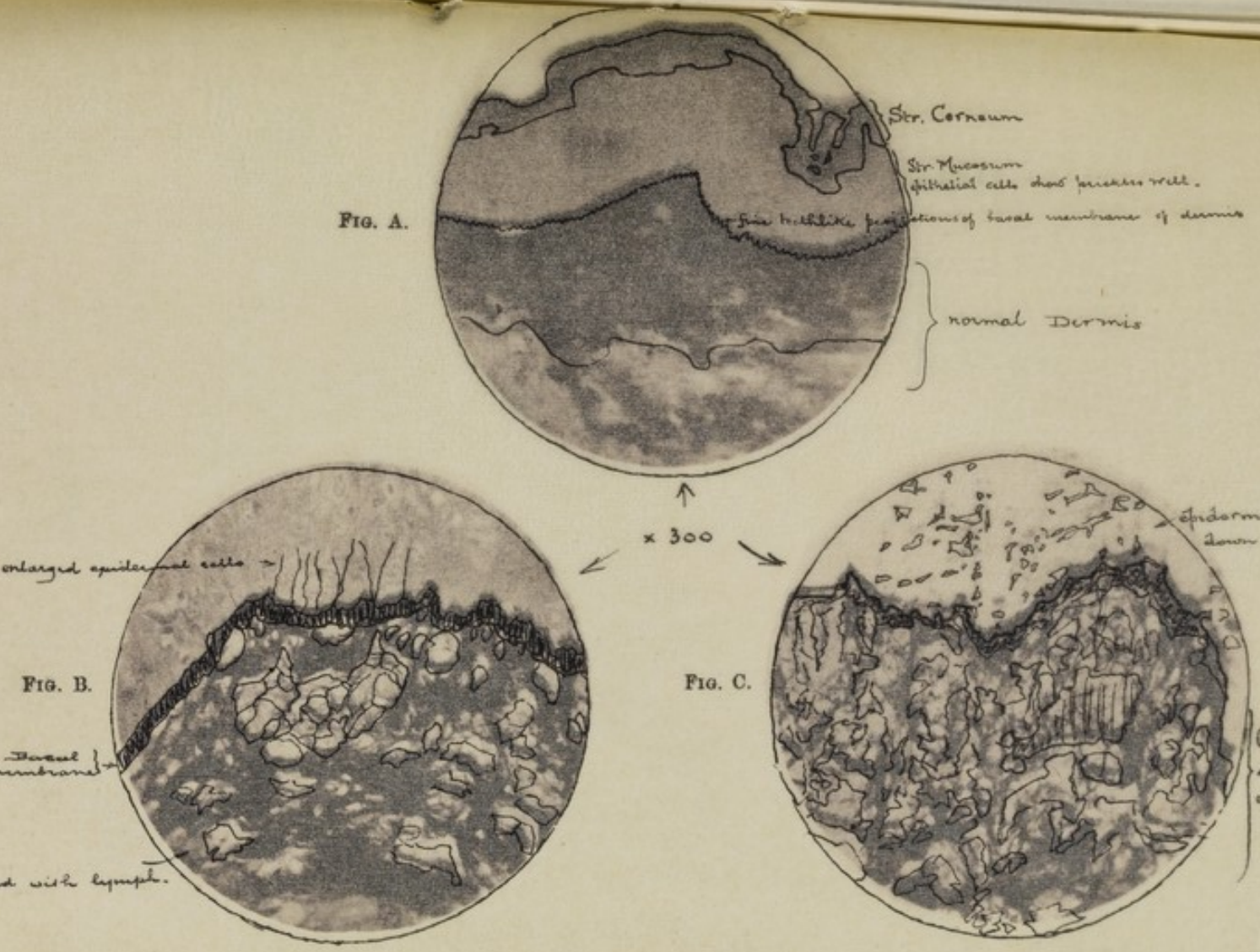


FIG. A.

FIG. B.

FIG. C.

PLATE XXXIII.

Figs. A.–C.

Vertical section of skin of calf 120 hours after vaccination. All three photographs are taken from the same section. Fig. A. is taken at the periphery of the section, and Fig. C. close to the line of inoculation.

[Magnified by 300.]