VI. *Research on the Smallpox of Sheep.* *By* E. KLEIN, *M.D., Assistant Professor at the Laboratory of the Brown Institution, London. Communicated by* JOHN SIMON, *F.R.S., D.C.L., Medical Officer of the Privy Council and of the Local Government Board.*

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SECTION I.—DESCRIPTION OR NARRATIVE OF EXPERIMENTS.

Experiments with fresh Lymph.

Experiment 1.—Lymph sent by Professor CHAUVEAU was used for the inoculation of a sheep on the 8th of December, 1873, in the following manner:—With the aid of a subcutaneous PRAVAZ syringe an extremely small quantity was introduced into each of four punctures in the true skin of the groin on the right side and of five on the left side. On the morning of the 13th of December, two of the punctures in the groin on the right side and four of those on the left were discernible as surrounded by a small circumscribed areola, which projected somewhat above the general surface; the puncture itself occupied the summit, and was marked as a brownish speck.

On the morning of the 14th of December there appeared a new pock on the right, and in the evening of the same day one on the left side. They increased rapidly in size, the red hyperæmic areolæ becoming larger in breadth and in thickness. After the first two days of their appearance (that is, after the evening of the 15th December) they only became more elevated, *i. e.* thicker.

As long as they increased in breadth they nearly all showed the central part most elevated; but as soon as they ceased to increase in breadth, or shortly before that, they became depressed and at the same time pale in the centre, whereas the peripheral part seemed now to be very much elevated and red; hence the line of demarcation between healthy and diseased skin was more marked than before.

In this stage they presented themselves as large patches above the general surface,

the peripheral part only being red. One puncture failed and one pock disappeared four or five days after its appearance; the others were excised, thus—

No. 1 on the 13th December.
„ 2 „ 14th „ in the morning.
„ 3 „ 14th „ in the evening.
„ 4 „ 15th „
„ 5 „ 16th „
„ 6 „ 16th „
„ 7 „ 21st „

Only No. 7 showed commencing pustulation.

The relation of the temperature of the animal was as follows:—

December	8 (before inoculation) . .	39°·3 C.
„	9	39
„	10	39
„	11	39·1
„	12	39·3
„	13	39·5
„	14	40·2
„	15	41
„	16	40·4
„	17	40
„	18	39·8
„	19	39·2
„	20	39·3

From this it appears that before the eruption of the variolæ the temperature rose only gradually; whereas it rapidly increased during the eruption and the increase of the pocks in size, and it became lower again as soon as they ceased to make any marked progress.

Experiment 2.—A second quantity of lymph from the same source as that used in experiment 1 was used in a similar manner. On the evening of January 15th, 1874, extremely small quantities of lymph were injected into the true skin of the groin of a sheep with the aid of a subcutaneous PRAVAZ syringe, four punctures being made on the right side and three on the left. On the evening of January 19th all the seven punctures were recognizable as circumscribed red elevations, the centre of which (the puncture) was marked as a brownish speck. All of these increased in size until January 24th, and while doing so they changed in the same manner as those in experiment 1: they became pale and depressed in the centre; whereas the periphery remained very much elevated above the general surface, and at the same time much reddened.

They were excised as follows:—

No. 1.	January	20
„ 2.	„	22
„ 3.	„	26
„ 4.	„	29
„ 5.	February	1
„ 6.	„	1.

One had disappeared.

Only Nos. 4, 5, and 6 showed traces of the formation of pustules.

The relation of the temperature of the animal was as follows:—

January 15	. . .	39·2° C.	
„ 16	. . .	39·2	
„ 17	. . .	39·3	
„ 18	. . .	39·5	
„ 19	. . .	40·3	
„ 20	. . .	40·8	
„ 21	. . .	41·2	
January 22	. . .	41° C.	
„ 23	. . .	40·7	
„ 24	. . .	41·1	
„ 25	. . .	40·8	
„ 26	. . .	40·5	
„ 27	. . .	41	
„ 28	. . .	40·8	

The temperature remained over 40° C. until the 1st of February, when the animal died. It had very extensive suppuration of the part from which the pocks had been cut out, and these extensive suppurations may easily have caused the abnormally high temperature after January 24. On a post-mortem examination being made, the lungs, liver, and peritoneum were found to contain numerous nodules of a parasitic nature, which of course stand in no relation to the inoculated disease.

Experiment 3.—On the 10th of March, 1874, lymph sent by Professor Cohn, of Breslau, diluted with ten times its bulk of thoroughly boiled ½ per cent. saline solution, was used for inoculating a healthy sheep; four punctures were made in the skin of the right and five in that of the left ear-lobe. On the 16th of March most of the pocks had made their appearance; they were in all respects similar to those described in experiments 1 and 2. They enlarged in size until March 20, and most of them became pale and depressed in the centre, thickened and red in the periphery. Commencing from the 24th they all showed suppuration and sloughing.

The course of temperature was as under:—

March 11	. . .	39·2° C.	
„ 12	. . .	39·3	
„ 13	. . .	39·2	
„ 14	. . .	39·3	
„ 15	. . .	39·5	
„ 16	. . .	40·5	
„ 17	. . .	40·9	
March 18	. . .	40·5° C.	
„ 19	. . .	40·4	
„ 20	. . .	40	
„ 21	. . .	39·8	
„ 22	. . .	39·8	
„ 23	. . .	39·6	
„ 24	. . .	39·5	

Experiment 4.—On the 1st of April, 1874, lymph diluted with 15 to 20 times its volume of saline solution was used for infecting a healthy sheep; thus

(*a*) Several punctures (three) were made in each ear-lobe.

(*b*) Four punctures in each mammary gland.

(*c*) Three in the right groin.

(*d*) About $\frac{1}{2}$ to 1 division of a PRAVAZ syringe was injected into the subcutaneous vein which runs between the mammary gland and the median line.

The pocks on the mammary glands, groin, and ear-lobes were discernible on the evening of the 4th of April as small red circumscribed swellings. On the 6th of April they were very much enlarged. On the 7th of April, while still increasing in size, they already showed a differentiation between a central somewhat pale depressed part and a peripheral portion still red and thickened.

On the evening of the same day (April 7) there appeared several small red swellings round the lips as the first indication of a general eruption.

The number of secondary pocks increased rapidly until the 11th of April, especially on the lips and nostrils; there were several in the axillæ, and a great number in the skin of the chest and hypochondrium.

During the appearance of the later secondary pocks those (secondary pocks) which had made their appearance first (on the lips) had already commenced to form pustules and to dry up.

It is important to state that the primary pocks, in this as in the former cases, were of a very much larger size than the secondary ones—many of the former reaching a diameter of $\frac{1}{2}$ to 1 or $1\frac{1}{2}$ inch, whereas the diameter of most of the secondary ones did not reach a quarter of an inch. There were, however, amongst the secondary pocks, especially those that came out very late, *e. g.* on the chest and hypochondrium, some which had a diameter of $\frac{1}{4}$ to $\frac{1}{2}$ inch, or even a little larger.

Another point worth noticing is this: the primary pocks showed in nearly all instances, while increasing in diameter, a differentation between a central depressed and a peripheral thickened part; whereas the secondary ones, except those that were of a large diameter, remained thickest in the centre, or at least did not become depressed. It will be seen subsequently that this difference is chiefly due to the differences in the changes of the epidermis.

The course of the animal's temperature was the following:—

March 31	39·1 C.	April 7	41·8 C.
April 1	39	„ 8	41·5
„ 2	39·1	„ 9	41
„ 3	39·3	„ 10	40·2
„ 4	39·8	„ 11	39
„ 5	40·8	„ 12	39·3
„ 6	41·8	„ 13	39·7

From the 14th of April the temperature was over 40° C.; this may be easily accounted for by the fact that, from this date for the next four or five days, a number of secondary pocks had been cut out from different parts, whereby extensive suppuration was produced in different places.

Besides the characters of the primary and secondary pocks above mentioned, they had all the common character, that when excised, no matter whether it was twenty-four hours after their appearance, or whether they were in the stage of increase of size, or of the formation of the pustules, or in the stage of drying up, the subcutaneous loose tissue by which the skin is connected with the subjacent adipose or muscular tissue was always found in a state of œdema. This œdema was greatest in the stage of their increase in size and for a short time after.

I have to tender my thanks to Mr. W. Duguid, Veterinary Surgeon at the Brown Institution, for assisting me in the experiments, and particularly for making and recording the observations on temperature.

Section II.—ANATOMICAL METHOD.

The pocks that were excised were all used for anatomical examination. The skin was invariably cleaned before the operation, and the pock was cut out with a surrounding small zone of healthy skin.

Immediately after the pock was cut out, clean instruments always being used, it was pinned out on a cork like a tent, the pins being fixed in the surrounding healthy skin, and the object was then placed, cork upwards, either in $\frac{1}{4}$ to $\frac{1}{5}$ per cent. chromic acid solution or in methylated spirit.

In some instances the pock was divided into two halves, and one half placed in each of the above-named reagents. After twenty-four hours the object was removed from the cork and returned to the hardening fluid.

Four or five days are usually quite enough to bring the object to such a consistence that it can easily, when imbedded in a mixture of wax and oil, be cut into microscopical sections.

Those pocks that were hardened in chromic acid were placed in spirit for several hours before they were imbedded.

It was found that chromic acid was preferable to spirit for hardening the pocks; for in those hardened in chromic acid the topography of the elements and their relation to each other was found to be unaltered and very clear and distinct; whereas when hardened at once in spirit it was found that these relations became considerably altered, the reagent producing too much shrinking.

As I shall afterwards mention, in all the pocks the corium was more or less œdematous; the hardening in spirit was found especially damaging in those pocks where there was only slight œdema of the corium.

The contrast between the microscopical preparations of that half of a pock hardened

in chromic acid and those of the other half hardened in spirit was very striking. In the latter case the œdema of the tissue of the corium could not be detected at all, whereas in the former the distribution of it and the changes of the elements of the tissue were very well preserved.

Section III.—Investigation of the organisms contained in fresh lymph by cultivation.

Previous Investigations.—In a paper by Professor Cohn, of Breslau, published in the 55th volume of Virchow's 'Archiv,' I find quoted the statements of the more important observers who have studied organisms in the fresh lymph of cow-pox and human smallpox.

Keber (Virchow's 'Archiv,' vol. xlii.) found in fresh lymph peculiar granular corpuscles which were perfectly different from pus-corpuscles; they are about $\frac{1}{300}$ to $\frac{1}{150}$ of a line in diameter, and contain 3 to 20 spherical elongated or hourglass-shaped particles, $\frac{1}{800}$ to $\frac{1}{3000}$ of a line in diameter.

After the solution of the cell-wall, those particles having become free, they are distributed through the lymph in enormous numbers, forming chiefly aggregates of 2 to 4 and 6 individuals, which are connected by a very delicate intervening substance. They divide rapidly into smaller and smaller particles. In old lymph (vaccine tubes) there are always present flakes and coagula, which consist of groups of the above-mentioned granular corpuscles, free particles and molecules held together by an intervening substance. These bodies represent the carriers of the virus. Keber could not, however, determine whether these particles are simply changed nuclei of the cells of the rete Malpighii of the epidermis, or whether they are living organisms.

Hallier and Zürn (Virchow's 'Archiv,' vols. xli. & xlii.) found in the lymph of cow-pox, sheep-pox, and human smallpox a swarming caudate *Micrococcus* of a conical shape endowed with a rotatory movement sometimes in the act of division.

Besides this they found delicate *Leptothrix*-filaments (*Micothrix*, Itzigsohn), in each small chain of which there was a distinct *Micrococcus*-swarm.

By cultivation Hallier thought to be able to transform the *Micrococcus* of the lymph into sporidia—further, into *Cladosporium*, *Sporidesmium*, *Tilletia*, *Monilia*, *Pleospora herbarum*, *Oidium*, *Eurotium*, *Aspergillus*, *Stemphylium*, *Ustilago*, *Torula*, and other forms, all of which he regarded as different stages of development of the *Micrococcus* of variola.

As the sporids originating from *Micrococcus* of sheep-pox develop, according to Hallier, in the air to a *Cladosporium*, which is identical with one of the forms of *Pleospora* recognized by Tulasne as the conidium-bearing form, and as this latter, a parasitical fungus living on *Lolium perenne*, is contained in spoiled hay, the inference which may be drawn from this is obvious according to Hallier, viz. that spoiled hay is the source of infection of sheep-pox.

Chauveau deduced the presence of organic particles being the carriers of the contagion

in the lymph of vaccine, as well as in that of sheep-pox, in a very ingenious manner from very numerous experiments (Comptes Rendus, vols. xlvii. & xlviii., February, October, and November 1868).

Burdon Sanderson, confirming the accuracy of the experiments of Chauveau, examined those particles microscopically, and found them to be identical with the *Torula* form of the *Micrococcus*, viz. small spheroids joining so as to form necklace-like chains. According to Sanderson, these spheroids (microzymes) tend to elongate into rod-like bodies endowed with a peculiar progressive or oscillatory movement, generally regarded as belonging to *Bacteria* (Twelfth Report of the Medical Officer of the Privy Council, 1869, p. 229). Cohn found that when lymph is collected from a pustule with the utmost care, it can be kept free from *Bacteria* or spores of fungi for an indefinite period. Cohn's method is as follows:—A perfectly clean lancet is used for the opening of the pustule; the drop of lymph which escapes from the aperture is drawn into a capillary tube, and then brought on a glass slide previously cleaned with ammonia, and covered with a covering-glass cleaned in the same manner, care being taken that there are no air-bubbles either in the middle or at the edges of the preparation. The edges of the covering-glass are then fixed by means of asphalt varnish, and the preparation can now be examined either fresh or after exposing it in an incubator to a constant temperature of 35° C.

In this way Cohn found that the lymph remains barren of *Bacteria* and any other germs of mould. Such clear lymph could also be used for inoculating, after Dr. Sanderson's method, boiled Pasteur's fluid without producing *Bacteria* or other vegetable fungi, even when it was kept exposed to a temperature of 30 to 40° C., whereas after the least contamination the fluid soon became turbid and decomposed. In the perfectly fresh lymph, Cohn describes, in accordance with Dr. Sanderson, pale spheroids of an extremely small size, below 0·001 of a millim.; they have no peculiar movement; they are, immediately after the preparation is made, generally isolated, occasionally in couples, like a dumb-bell.

In a very short time, however, the dumb-bells increase in number, and form curved or zigzag chains of four members. After one to two hours there are already numerous necklaces of eight members, or the members arrange themselves like *Sarcina*, or they form, by simple juxtaposition, groups or colonies.

The spheres proliferate very quickly by transverse division; so that after six or eight hours there are, besides chains of two to four and eight members, also very numerous colonies of sixteen to thirty-two or more members to be found all over the preparation.

The proliferation continues during several days; the colonies enlarge and reach even the size of ten micromillimetres.

A colony or zooglœa represents a group of spheres held together by an intervening gelatinous transparent substance. Cohn calls these organisms *Microsphæra vaccinæ*, and places them amongst the family of Schizomycetes in the group of Bacteriaceæ (Virchow's 'Archiv,' vol. lv. p. 234). In the second volume of 'Beiträge zur Biologie der Pflanzen,' in his well known "Untersuchungen über Bacterien," p. 161, Cohn calls them *Micrococci*

vaccinæ; this *Micrococcus*, as well as any other *Micrococcus*, i. e. *Sphærobacterium*, differs completely from HALLIER's *Micrococcus*, as the former stand in no genetical relation whatever either to other kinds of *Bacteria* or to the spores of other fungi with a mycelium.

I come now to describe the results of my own observations of the lymph of variola of sheep. Clear lymph, which had been kept for several days in a sealed capillary tube, was diluted with thoroughly boiled half per cent. saline solution and was used thus: one portion of the diluted liquid having been reserved for further experiments, the remainder, which was intended for microscopical examination, was sealed, immediately after it was prepared, with dammar varnish and examined. It contained structures as represented in Plate 29. fig. 1. First there were to be found minute highly refractive spheres isolated, or in couples or in small groups; they correspond to the solid granules (*Micrococci*) in COHN'S figure in the above-mentioned paper. They did not show any other than Brownian movement. Then there were present a great number of circular pale bodies, which, from their circular shape and size, could be easily recognized as decolorized blood-corpuscles. They were generally to be met with in small groups, between the members of which the same spheres, *i. e.* dark granules as before mentioned, were seen in couples or in necklace-like chains; these *Micrococci* followed exactly the interstices between the blood-corpuscles. Besides these structures there were to be seen a few rod-like *Bacteria* belonging to those types which are designated by COHN as *Bacterium termo* and *Bacillus subtilis*.

They were either isolated or in couples, and exhibited only slight oscillatory movement. The most characteristic features, however, were the following:—

(*a*) Lumps of a pale transparent substance containing very irregularly distributed smaller and larger granules, the smaller granules being pale and indefinite, the larger ones very bright and highly refractive.

(*b*) Spheres generally considerably larger than the spheres above mentioned, at least twice as large. They were arranged in small groups, chiefly composed of couples or of necklace-like chains.

These spheres were different from the above-mentioned ones, not only in their being larger, but chiefly by the fact that they were bordered by a sharp line as if by a membrane, whereas their contents appeared perfectly transparent. They correspond to the spheres figured by SANDERSON, and to the spheres (transparent) in COHN'S figure, only that they are generally larger than the dark solid granules, and not of equal size as represented in COHN'S figure.

(*c*) Groups consisting of the highly refractive small spheres above named and the granules mentioned under (*a*). They are also represented in COHN'S figure in VIRCHOW'S 'Archiv,' with the difference that the transparent spheres are always larger than the solid highly refractive ones. On careful examination of these groups of mixed spheres, it is found that there are all transitional forms between the two kinds of spheres which

form the groups, viz. small spheres not markedly larger than the highly refractive ones, the centre of which is different from the peripheral part, the former being transparent, the latter a highly refractive substance: then there are others whose central transparent part is greater, the highly refractive substance representing only an envelope, generally possessing at one spot a thickening; these spheres are markedly larger than the solid granules, and smaller than the perfectly transparent spheres above mentioned: further, there are others that are still larger, and whose highly refractive substance is reduced to a very thin envelope, possessing at the same time at one point a minute granule. It is quite evident that these are transitional stages.

If the preparation is kept for twenty-four hours in the incubator at a temperature of about 38° C., the number of transitional spheres is immense; they are either isolated or form couples with each other or with one solid or one perfectly transparent sphere.

(*d*) Very bright shining highly refractive spheres, which are not only of a characteristic brightness and somewhat greenish in colour, but which appear at least of twice the size of the first-mentioned dark granules. They are found to form small groups, chiefly composed of couples, which resemble rod-like structures with terminal swellings, the more so as there is a more or less distinct connecting substance between the two joints. Some of these couples appear to be surrounded by a narrow clear zone limited by a thin membrane. There are also isolated bodies of this kind to be seen, which appear to be in the act of division, viz. a somewhat elongated sphere of the same bright substance showing a slight constriction in the centre.

If the preparation has been kept for twenty-four hours in the incubator, the above-mentioned pale transparent masses containing irregularly distributed granules are seen to undergo some remarkable changes. They become more or less distinctly fibrillar, in such a manner that they appear to consist of a feltwork of very delicate branched filaments, in or on which the granules are now found. Plate 29. fig. 2 gives a very accurate representation of them. Under a very high power (such as an immersion-lens) we see that these masses consist of spherical bodies, granules of different sizes arranged in rows: the members of each row are imbedded in, or, rather, connected by, a pale transparent substance; hence the appearance of minute granular fibrils. In some places the granules seem to lie only alongside the fibrils. Still later (forty-eight hours) the network of fibrils is very distinct, especially because the large masses, after having enlarged considerably, are seen to break up into smaller masses, in which it is easier to trace the individual fibrils.

The granules have increased considerably in size; and now it is very easy to recognize that they correspond completely to the spheres above mentioned as (*b*) and (*c*); that is to say, that the highly refractive spheres (the granules) become gradually transformed into transparent spheres bordered by a delicate membrane, and that all these spheres bud on, and become separated from, the filamentous matrix.

The longer a period the preparation is subjected to the constant temperature, the more numerous highly refractive and transparent spheres originate from that matrix.

Spheres in the act of transverse division are very often met with.

It is worth noticing that the few rod-like *Bacteria* mentioned above as being present in the fresh preparation disappeared completely after the preparation had been kept in the incubator twenty-four to forty-eight hours.

A drop of lymph was obtained on March 24 from a pustule of an animal which had been infected March 10 (see experiment 3), and was used for a microscopic preparation as in the former case, without, however, being diluted with saline solution. When examined fresh, it showed, besides large numbers of granular pus-corpuscles and coloured blood-corpuscles, numerous small highly refractive granules, isolated and in couples, exhibiting molecular movement. The preparation was placed in the incubator and kept at a constant temperature of 32° C. for twenty-seven hours, after which time when examined it showed the following structures:—

(*a*) Besides intact granular pus-corpuscles there were numerous pus-corpuscles the substance of which had become swollen and transparent; these contained two to six spherical homogeneous, not very highly refractive, bodies, about half the size of a coloured blood-corpuscle, or even less. Some pus-corpuscles containing these bodies were seen to be in the state of becoming disintegrated, and thus those spherical bodies becoming freed. That they are not nuclei of the pus-corpuscles is shown by the fact that they become the more distinct the more the matrix of the pus-corpuscle becomes swollen and disintegrated. They are most distinct when they have become freed from a corpuscle. Besides they have a *slightly greenish colour* and are homogeneous; whereas it is well known that when pus-corpuscles swell, also their nuclei become swollen, and have then the appearance of vesicles bordered by a thin membrane. Similar spherical bodies are found in the surrounding medium in great numbers; they are either isolated or in couples; they are generally spherical; occasionally they are oblong, and possess a more or less deep constriction in the middle part.

(*b*) From these forms one can trace others, which possess one or two small dark granules; in the latter instance the corpuscle is generally somewhat elongated, and the grannules are situated at its pole.

From these, again, we come to other forms, which consist of two granules (dumb-bell) surrounded by a very thin pale envelope, and, finally, dumb-bells in which there is just a trace of the envelope to be seen under a very high power. I refer the reader to 1 in fig. 3, Plate 29, in which most of the forms just mentioned are represented.

From this we are justified in saying that there exist spherical bodies, either enclosed in pus-corpuscles or freely suspended in the medium, which are not nuclei; they are isolated or in couples (transverse division), of a slightly greenish colour, homogeneous, and pretty nearly of the same size.

It may be further stated that these spheres become transparent, while in them granules, *i. e.* highly refractive minute spheroids (*Micrococci*), make their appearance; these multiply by the act of transverse division (dumb-bells), and the matrix now represents a transparent more or less distinct envelope or connecting substance of the dumb-bells.

(*c*) That there is going on in these dumb-bells an immensely rapid proliferation by transverse division is proved by the really astounding number of *Torula*-like chains (necklaces), most of them consisting of 4, many of 6, 8, 12, and 16 members (see 2 in fig. 3). All the *Micrococci* of these necklaces are distinctly held togther by a transparent connecting substance. The larger the necklaces grow, the more they become curved and convoluted (see 3 in fig. 3).

It is worth noticing that in some instances the necklaces possess at one end or laterally at one point a comparatively large pear-shaped body, which consists of a greenish matrix, in which there is occasionally a highly refractive *Micrococcus* to be seen.

The necklaces, which have grown to an immense length, and which have become convoluted in a very complicated manner, are very liable to break up into a number of shorter chains; in this case we have a convolution of necklaces from which several free ends stick out.

(*d*) If in such a convolution, consisting of a single chain or of several of them, the *Micrococci* become more and more closely packed together, and the connecting substance of the individual chains becomes more and more coalescing, then we have a colony of HALLIER or a zoogloœa of COHN.

These changes of chains into colonies can be traced with great ease.

(*e*) There are many colonies which can be still recognized as being composed of necklaces closely packed together, and from which project shorter or longer filaments—in some places showing distinct divisions into rod-like joints, in others apparently smooth and homogeneous. I have tried to reproduce these features in fig. 3, 4, as accurately as possible.

If the preparation is left a further twenty-four hours in the incubator and then examined, it is found that the isolated necklaces and colonies increase in number and size, whereas at the same time some of the *Micrococci* of the latter appear to become not only larger, but of a very great brightness and somewhat greenish. At the edges of the colonies, where the latter happen to project freely in small groups, we find them to possess a striking resemblance to those bodies represented in Plate 29. fig. 1, 7.

At the same time we find a great number of perfectly transparent spheres, exactly similar to those described in the first preparation, and represented in fig. 1, 2, and fig. 2, 1, as far as size and aspect is concerned.

They can be easily traced as being transformations of the spheres described on page 224 and represented in fig. 3, 1. The preparation having been kept in the incubator until March 28, *i. e.* during four days, was examined again, and it was found that the number of colonies was very great, that many of the *Micrococci* had become enlarged and of great brightness and of a greenish aspect. Besides, the filaments represented in fig. 3, 4, appear now to be very distinctly composed of rod-like joints, many of which have a more or less distinctly granular aspect.

I have until now deliberately abstained from introducing any terms excepting "*Micrococci*" and "colony," and I have tried to limit myself to a simple description of what

I found and what I think I am justified in connecting with each other; and if I compare these observations with those of other authors, I am inclined to believe:—

(1) That the spheres figured by SANDERSON, and some of those figured by COHN, being identical with those figured by me, fig. 1, 2, and fig. 2, 1, do not represent the true *Micrococcus* of the lymph of variola in its active condition, but represent rather a dropsical condition of the true active *Micrococcus*, which is a highly refractive spheroid, and appears solid and uniform under the microscope.

(2) That the filaments stand in a genetical connexion with the development of *Micrococcus* is shown by the observation of the lymph described on page 223, and represented in fig. 2, 1, 2, & 3.

SECTION IV.—ANATOMICAL INVESTIGATION OF THE ERUPTION.

A. *Summary of previous Investigations.*

According to LUGINBÜHL* the pathological process in human smallpox consists in the penetration of the *Micrococcus*-variolæ into the skin, partly through the epidermis, partly through the hair-follicles and sweat-glands. By this means the inflammatory process known as smallpox, characterized by the following anatomical changes, originates:—

1. In the epidermis an opaque swelling (VIRCHOW) takes place, due to the cells of the rete Malpighii containing *Micrococci*. The nuclei of some of the epithelial cells, as well as some of the latter, become dropsical. The cells of the rete Malpighii, which are filled with *Micrococci*, show active proliferation; they enlarge and their nuclei divide rapidly. Thus multinuclear giant cells are formed which are crammed full of *Micrococci*. In the deeper strata of the rete Malpighii, where the cells have only a very delicate cell-wall, the giant cells never become very large; their membrane soon bursts and their nuclei become free: in the more superficial strata, however, the cells possess thicker and more resistant walls, and therefore the proliferation of their nuclei may go on for a much longer time.

By the giant cells and those groups of nuclei just mentioned, as well as by the dropsical epithelial cells, spaces are formed in the rete Malpighii which represent the pustules. Certain conical giant cells in the deeper strata of the rete Malpighii, while growing towards the corium, cause the communication of the latter with the spaces in the epidermis; in this way cellular elements penetrate from the epidermis into the corium, whereas their previous place is occupied by a pale coagulable fluid. If the communication between epidermis and corium is once established, then the formation of the pustules makes rapid progress; all around them appear new giant cells, which, however, do not reach a large size, but soon lose their former contents (*Micrococci*), and in its place contain fibrinous coagula; the neighbouring cells become compressed and dragged in manifold ways, so that finally a system of spaces is found, separated and penetrated by lumps (giant cells) and tracts (compressed epithelial elements).

* "Der *Micrococcus* der Variola," Arb. aus dem Berner path. Institut, 1871–72, p. 159.

In the sweat-glands and hair-follicles the epithelial cells show also the opaque swelling due to their containing *Micrococci*.

Between epidermis and corium of those places where there is the least microscopical change there are constantly semilunar spaces of different diameters to be seen, the convex surface of which corresponds to the elevation of the papillæ. These spaces contain *Micrococci* imbedded in a transparent fluid. In those pocks in which the formation of pustules is going on, the corium becomes gradually penetrated by the elements which have been previously in the epidermis, viz. *Micrococci* and nuclei; thus the boundary between epidermis and corium gradually loses its sharpness. The papillæ show sometimes in their longitudinal axis an infiltration of fine granules.

In the deeper parts of the corium larger or smaller tracts present themselves, formed partly by larger spherical cells and partly (seldom though) by a finely granular substance. LUGINBÜHL therefore makes the whole process start in the epidermis, and hence gradually to extend into the corium. These statements are, according to my observations of variola of sheep, wholly inconsistent with the facts occurring in this latter disease, which, as is well known, is in clinical and anatomical respects very similar if not analogous to smallpox of Man.

The results of the examination of smallpox of Man obtained by AUSPITZ and BASCH are described by NEUMANN*, who confirms fully the observations of these authors, in this way:—In papules, on the second day of their appearance, the epidermis is elevated, apparently from the increased thickness of the rete Malpighii, the individual cells of which are larger than those of the neighbouring normal parts; their nuclei are enlarged. The vessels in the corium, those in the papillary region as well as those beneath it, are distended; on their walls are numerous small roundish cells, and similar cells are found in the stroma of the papillæ. The papillæ and glands are unchanged.

The structure of the vesicles and the pustules is thus described:—Under the stratum corneum of the epidermis there is a layer of longitudinal cells, which merge uninterruptedly into the roundish or flattened and distinctly swollen cells of the rete Malpighii; beneath this a meshwork is seen, which is nearer to the stratum corneum than to the corium, and occupies a great part of the breadth of the vesicles, but does not extend deeply. This mesh-like structure consists of transverse septa of fine fibrous tissue, which are evidently formed of the compressed spindle-shaped cells of the hypertrophied rete Malpighii; in its interstices pus-cells are imbedded, some of the large vesicles containing ten or more small cells. Under this mesh-like structure and extending between the papillæ there are found roundish cells, which either resemble those of the meshwork or the swollen Malpighian cells. The underlying papillæ appear broader, and those in the immediate vicinity of the vesicles lengthened. Proliferation of cells is observed around the vessels.

The meshwork extends gradually towards the corium, and increases in width from the centre to the periphery; in the interstices there are round cells.

* See 'Textbook of Skin Diseases,' by Dr. NEUMANN, translated by Dr. PULLAR, London, 1871, p. 74.

The pustular contents are enclosed, as if by a capsule, by two layers of unnucleated epidermic cells. Besides the pus-cells there are also unnucleated elements (insoluble in acetic acid) with fine granular contents.

Auspitz and Basch are of opinion that the so-called umbilicus of the pock, the central depression, is due to the pustule gradually extending towards the periphery, whereby the pressure in the centre diminishes and the central part becomes depressed.

According to Rindfleisch and others, on the other hand, the central umbilicus is produced by there being a sweat-duct or a hair-follicle in the centre of the pock, which, when the pustule is formed in the epidermis, keeps the latter fixed to the corium like a retinaculum*.

B. *Anatomical Peculiarities of the Skin of the Sheep.*

Before I describe the pathological changes of the skin, I will draw the attention of the reader to several anatomical points, as regards the structure of the skin of the sheep, which have not been properly described yet.

(*a*) In the skin of most parts of the body (*e. g.* groin, wall of abdomen and chest, axilla, and so on) the epidermis (stratum corneum plus rete Malpighii) is in hardened preparations thin and rather opaque—only the deepest, or at most this latter and the next stratum, appear to be composed of cells elongated vertical to the surface; the other layers of the rete Malpighii are composed of more or less polyhedral cells, which are the more flattened the nearer to the surface. In general the outlines of the cells are very indistinct; the whole rete Malpighii looks more like an opaque granular substance, in which nuclei are imbedded in more or less definite intervals.

(*b*) The papillæ of the corium are very scarce, short, and small; in many places the rete Malpighii rests on a corium, the surface of which is only slightly wavy, *i. e.* the papillæ are only just indicated.

(*c*) The corium may be divided into a superficial stratum, which includes the papilla and the tissue directly underneath it, and a deep stratum beneath the former, containing the sebaceous glands, sweat-glands, and the roots of the hair-follicles.

The superficial stratum is a dense connective-tissue feltwork with numerous elastic fibres; it contains the ultimate ramifications of the blood-vessels and lymphatic vessels.

The deep stratum is somewhat looser in its structure than the former, but is still similar to it, as the connective-tissue bundles of its matrix are very small, run in all

* If the central depression I have mentioned in the primary and some secondary pocks of sheep in Section I. correspond to what is described as the umbilicus of variola of man (and to all appearance they do correspond to each other), then I must anticipate so far as to say that this central depression has no connexion whatever either with the formation of the pustule or with the hair-follicles or sweat-ducts, but, as we shall see hereafter, is due, to a great extent, to certain morphological changes of the epidermis in the centre of the pock. In Luginbühl's paper, quoted above, I find, on p. 160, a reference to Auspitz and Basch, Neumann, and Cornil having found *Micrococci* in the meshes of the fully developed pustule and also in the corium; and, finally, C. Weigert describes (Centralblatt der medicin. Wissensch. 1871, No. 39) sinuous tubes in the corium of smallpox, which tubes (lymphatic vessels) are filled with *Micrococci*.

directions, and cross and join each other very closely. This stratum contains, in that layer which is nearest to the surface, the sebaceous glands, a little deeper the roots of the hair-follicles, and in the deepest layer the sweat-glands.

(*d*) The subcutaneous tissue between the corium and the panniculus adiposus may also be divided into two strata, a superficial and a deep one. This superficial stratum is very markedly different from the deep stratum of the corium; for it consists of large broad bundles of connective tissue, which run in two, or generally in three, directions; they are by no means so close as in the latter, and the interfascicular spaces are therefore more distinct and much larger. The amount of elastic tissue is not great.

This stratum contains the minor trunks of the blood-vessels and the lymphatics, which run to or from the corium; they are not very numerous.

The deep stratum of the subcutaneous tissue is similar in its structure to the former; it is still looser, and contains the main trunks of the blood-vessels and lymphatic vessels, and also a greater or smaller amount of fat-tissue, either in the act of development or already fully developed.

(*e*) The sebaceous glands are characteristic for being enormously large in respect to the hair-follicles into which they lead; they are short, slightly branched tubes, swollen at their end, and lead into a large duct, which is identical with the mouth of the hair follicle. The sebaceous glands of the groin and the axilla are the largest. The hair-follicles are possessed of arrectores pili, which, relatively to the size of the hair-follicles, are of very great strength. When, after an oblique course around the sebaceous gland, they enter the superficial stratum of the corium, they split in two, three, or more minor bundles, which can always be traced very close to the papillary layer, into which they do not, however, enter, but generally bend before that into a horizontal direction.

Each sweat-gland consists in its secreting part of a tube, which is generally convoluted in a direction parallel to the surface. The tube consists of an apparently homogeneous thick membrane, on the inner surface of which lies one or even two continuous layers of longitudinal unstriped muscles. Close to these, without the intervention of a membrane, is attached a single layer of nucleated columnar epithelial cells, which line the lumen of the gland.

The duct of each gland becomes narrower the more it approaches the surface, and pursues always an almost vertical course; it leads generally into an epithelial prolongation of the mouth of the hair-follicle, or, as one might say with equal truth, into the mouth of the duct of a sebaceous gland. The duct of the sweat-gland does not possess a muscular coat, at least not in some distance from the proper gland, and is lined by polyhedral epithelial cells, which become the more laminated (stratified) the nearer to its mouth. The lumen of the sweat-duct is much narrower than that of the proper gland, and is reduced at its mouth to its smallest diameter *.

* The sebaceous glands of the lips of the mouth are different from the sebaceous glands above described, the former (glands of the lips) being very much longer; they consist generally of a number of narrow ducts leading into a common hair-follicle. They are of a relatively great length, and terminate in a number of wavy, pear-shaped, somewhat branched sacs.

(*f*) The large lymphatic trunks, situated, as mentioned above, in the deep stratum of the subcutaneous tissue, are provided with valves; they form rich anastomoses, and stand in direct connexion with the intercommunicating system of the interfascicular spaces, *i. e.* lymphatic spaces of the deep and superficial stratum of the subcutaneous tissue. The interfascicular spaces contain the connective-tissue corpuscles; that is to say, the latter are so fixed upon the connective-tissue bundles that the interfascicular spaces appear to be lined by the connective-tissue corpuscles. The deep stratum of the corium contains the greatest number of lymphatic vessels; they have chiefly a course parallel to the surface; they are provided with valves, and stand everywhere in direct connexion with interfascicular spaces lined by the connective-tissue corpuscles. The lining membrane of the lymphatic vessels, composed of a continuous layer of endothelium, is therefore in direct continuation with the latter. The interfascicular spaces of this and of the superficial stratum of the subcutaneous tissue are in many places very much enlarged, and resemble lymphatic sinuses or lymphatic sacs, through which, in many instances, the smaller branches of blood-vessels are seen to penetrate—perivascular lymphatics. These lymphatic sinuses are especially well developed around the sebaceous glands at the bases of the arrectores pili, and also around the proper sweat-glands. Into the plexus of lymphatic vessels which lie in the deep stratum of the corium lead lymphatic vessels which come from the superficial stratum of the corium; they are also provided with valves, and can be traced up to the papillary layer. Many of the lymphatic vessels of the superficial and deep stratum of the corium are seen to be in close relation to the blood-vessels, especially the veins, as they always seem to run with each other.

C. *Early Stage of the Development of the Primary Pustules.*

As has been already stated, the pocks designated as primary were excised in their different stages of development up to the period of pustulation, for anatomical examination. The latest stage (*stadium exsiccationis*) has not been particularly investigated, as it does not differ from any other restitutory process of the skin. In examining pocks in the earlier stages, I have usually cut up the whole into microscopic sections. Of the more advanced pocks I have examined sections of only one half.

Sections through the primary pocks which had appeared only several hours (six to twelve) show as the most characteristic features the following:—The epidermis has markedly increased in thickness, chiefly due to an increase of the thickness of the rete Malpighii. The cells of the latter are more transparent and larger, their outlines more distinct than in the normal condition; the cell-substance is finely granular; the nuclei are enlarged, each possessing one or, more generally, two distinct nucleoli. The difference in size, distinctness, and transparency of the rete Malpighii is very striking at the point where the skin of the pock is in contact with healthy skin. The cells of the three deeper strata of the rete Malpighii are elongated in a direction vertical to the surface. The papillæ are much more distinct in the corium of the pock than in that of the neighbouring healthy tissue; they appear broader and longer. The blood-vessels of

the corium are somewhat distended; in the small veins and capillaries the endothelium is seen with much greater distinctness than in those of the healthy tissue. Especially in transverse sections through small veins and capillary blood-vessels it is found that the endothelial cells are swollen, distinctly granular, and their nuclei enlarged. Accordingly the wall of these blood-vessels appears thicker and altered. This is in so far an interesting fact, as COHNHEIM, by the aid of experiments (see his latest researches on Inflammation, Berlin, 1873), arrived at the conclusion that the walls of the blood-vessels must necessarily in inflammation undergo some changes to cause the exudation of the fluid and the formed parts of the blood, which, as is well known, represent very material morphological symptoms in inflammation.

The tissue of the corium in general is slightly œdematous, the interfascicular spaces, *i. e.* lymph-canalicular system, being larger and more clearly visible than in the normal condition. The connective-tissue corpuscles, situated in the interfascicular system of spaces, are recognizable at the same time in many places, their nuclei being more distinct than in the normal parts. In the interfascicular lymph-canalicular system of the corium, chiefly where the blood-vessels are more numerous, *e. g.* around the glands, there are found lymph-corpuscles, which are the more numerous the nearer one approaches to the blood-vessels; this fact enables us to say that they are probably all extravasated colourless blood-corpuscles. Whereas the lymphatic vessels of the corium are hardly to be found in the corium of healthy skin after simple hardening, they are in our case easily traced, being distended and more or less filled with a transparent, homogeneous or finely granular substance, which in all its appearances resembles coagulated plasma.

The changes of the subcutaneous tissue are similar, only much slighter; they diminish more and more towards the depth. We have therefore only such changes as one might expect in inflammation in the strictest sense of the word, viz. distended blood-vessels, altered walls of blood-vessels, exudation of plasma, and extravasation of colourless blood-corpuscles, seen in the distended lymph-canalicular system and distended lymphatic vessels. The greater distinctness of the connective-tissue corpuscles and the enlargement and greater transparency of the rete Malpighii are probably due to the increased irrigation of the tissue with exuded plasma.

If we direct our attention to the pocks that have been cut out twenty-four hours after they made their appearance, we find the changes above stated much more marked.

The rete Malpighii is still thicker, more transparent, the nuclei of the epithelial cells of the deeper strata enlarged, many of them in the act of division or already divided; the papillæ and the corium in general more œdematous than in the former case, the lymph-canalicular system being not only very marked and distended, but containing a finely granular material—coagulated plasma. Further, the infiltration of the corium with lymph-corpuscles has increased, it being now possible to trace the course of these bodies from the larger branches of the blood-vessels of the corium into the distended lymph-canalicular system. The connective-tissue corpuscles of the œdematous corium appear

now swollen, distinctly granular, and their nucleus in the act of division or already divided. The changes of the walls of the blood-vessels, as stated above, are now to be traced not only in the blood-vessels of the corium, but also in those of the superficial parts of the subcutaneous tissue. Although the œdema and infiltration with lymph-corpuscles is chiefly limited to the corium, still it is noticeable that the subcutaneous tissue has in some places, especially around larger blood-vessels, become also materially involved in the morbid process, the interfascicular spaces being in some places very markedly distended, and containing not only a few lymph-corpuscles, but also a finely granular material, in which are imbedded peculiar ovoid or spherical sharply outlined structures, containing in a clear substance one large or two, three, or four small highly refractive bodies. These structures are of different sizes, the smallest being not smaller than the nucleus of a connective-tissue corpuscle, the larger ones two or three times as large. The connective-tissue corpuscles which line the bundles of the connective tissue are at the same time enlarged; they appear swollen, granular, and their nucleus single or divided.

Comparing the sharply outlined structures found in the interfascicular spaces, as mentioned just now, amongst themselves, one cannot help thinking that the highly refractive bodies found in their interior are in the state of undergoing proliferation by division.

I will draw the attention of the reader to Plate 32. fig. 12, which shows these relations very accurately. The question now arises, What are these sharply outlined structures with the highly refractive bodies in their interior?

That these bodies are free nuclei must be excluded at once,—first, free nuclei not being observable anywhere else in the tissue ; and, secondly, the nuclei of connective-tissue corpuscles and of lymph-corpuscles being altogether different. The issue rests only between their being lymph-corpuscles (extravasated) or something not belonging to the skin at all; I mean, a fungus. In case they should be lymph-corpuscles, the transparent sharp-outlined matrix would represent the swollen cell-substance, and the highly refractive bodies in their interior would correspond to the shrunken coagulated nuclei. It certainly cannot be denied that pus- or lymph-corpuscles, when treated with dilute acids (*i. e.* chromic acid, acetic acid), show appearances similar to those just mentioned; but in the present instance, although the preparation in question had been hardened in chromic acid, no such appearance was to be found either in the lymph-corpuscles situated in the corium or in the veins and lymphatic vessels of the subcutaneous tissue. In these parts the lymph-corpuscles appeared as they do in general in hardened preparations, viz. as spherical more or less transparent or finely granular cells, containing one relatively large nucleus, and seldom two or three small spherical nuclei which are readily stained with carmine. The structures in question not only differ from lymph-corpuscles in shape, but they also show a great difference in their contents—the contents of the former being highly refractive bodies in the state of division, which are not stained with carmine at all.

From all this it is probable that they are not lymph-corpuscles; and therefore it is

not impossible that they do not belong to the tissue of the skin itself, but that they perhaps correspond to what HALLIER calls *Cryptococcus*. This is also supported, to a certain extent, by the fact that I found them chiefly in those parts of the subcutaneous tissue which (as I could make out from several circumstances) had been penetrated by the canula of the injecting-syringe when the inoculation with lymph was performed.

The most important characteristics of this stage of the disease, however, are those which depend on the changes in the lymphatic vessels in the deeper part of the corium. The lymphatic vessels (those accompanying blood-vessels and filled more or less with lymph-corpuscles, as well as the others) are, as stated above, distended, and from this reason, as well as from the lymph-canalicular system being also distended, are distinctly seen to be in direct continuity with the latter, viz. with the interfascicular lymph-canalicular system.

Many of these lymphatics contain a material which, as represented in Plate 31. figs. 9, 7, & 8, is composed of a transparent matrix, in which lie imbedded highly refractive spheres, in some parts closer than in others.

From the lymphatics this material extends also into the interfascicular lymph-canalicular system, or, more correctly speaking, it extends from the latter into the former. In some lymphatics these highly refractive granules are seen to be arranged in shorter or longer, branched or unbranched filaments. These filaments are more or less curved, and resemble either necklaces or smooth filaments according as the individual joints are more like spheres or like rods. One and the same filament may also be partly granular, *i. e.* like a necklace, and partly smooth.

In those places where they are to be found in greater numbers they are seen to cross each other and decussate, so as to form a close feltwork.

These relations come out very distinctly in a little later stage. Thus in a pock cut out a little after forty-eight hours (see Plate 31. fig. 9), it is seen that in the lymphatics of the deeper stratum of the corium the granular mass is not only replaced by filaments (or, let us say at once, that most of the granules have arranged themselves into filaments), but, what is also of great importance, the filaments have more or less lost their granular aspect, and have become smooth, longer, and more branched.

In fig. 10, I., a lymphatic vessel of the subcutaneous tissue is shown, in which is seen a network of branched filaments without granular matter, some of which exhibit small swellings at one or other point of their course, while in other instances the swelling is at the end of the filaments.

There can, I think, be no doubt whatever that the granular material is not plasma or serum coagulated by the hardening reagent; for these granules are not only of a definite large size (very much larger than the granules one meets with in coagulated serum), but stand, as the examination proves, in a definite genetical relation to the filaments. As regards these latter there is only one possible explanation, viz. that they represent an organism.

In pocks which were cut out between twenty-four and eighty-four hours, all the

distended interfascicular lymph-spaces, and especially the distended lymphatic vessels of the corium (in the lymphatic vessels of course with much greater distinctness), contained these filaments in very great numbers. In the more advanced stage of their development the individual filaments differ from each other considerably in size; the branching is more distinct, so that they form a network. Each filament follows a course which is alternately curved and straight, so as to form more or less dense convolutions. The filaments are highly refractive, and appear to be of a greenish colour, which is the more distinct the thicker they are. Generally they appear perfectly smooth, even under a high power; in some instances, however, I was able to see not only that they were composed of elongated joints, but also that it was possible to distinguish a surrounding delicate membrane and highly refractive greenish contents. The composite nature of the filaments is especially noticeable at the free ends; here it can be distinctly seen that they are composed of joints. Towards the ends of the filaments these joints are short, elliptical, or spheroidal, becoming more and more elongated as their distance from the end increases. There are terminal filaments of a relatively great length, which are distinctly composed of spherical or elliptical joints. Other filaments occur which, at some point near the free end, possess one or more elliptical or spherical joints larger than the rest, or give off small lateral branchlets terminating in a similar manner. In the necklace-like filaments so formed it is sometimes observed that each of the spherical or elliptical bodies just mentioned appears to be enveloped by a delicate thin membrane (*cf.* Plate 31. fig. 10, II., III., IV., & V., and Plate 32. fig. 11, A & B).

In fig. 11 it will be noticed that the spherical bodies undergo transverse division, thus forming dumb-bells and necklaces, and also that, by becoming elongated, they form the basis of the filaments.

I think from the foregoing it can be stated with safety that we have to deal with the development of a fungus. It commences as a mass which corresponds in appearance to a mass of *Micrococci* (*i. e.* zoogloœa); these grow into a mycelium, the constituent filaments of which differ very considerably from each other in thickness. The mycelium fructifies, forming conidia like an *Oidium*. The spherical spores proliferate by transverse division, forming thus smaller groups—dumb-bells and necklaces of smaller and smaller bodies. The conidia are, just like the filaments of the mycelium, highly refractive and of a greenish colour.

The deeper stratum of the corium is the part in which the fungus is found chiefly in the earlier stages of the disease.

Eventually the superficial stratum of the corium, as well as the superficial stratum of the subcutaneous tissue, is filled with them; the former to a greater extent than the latter. As the natural direction of movement of liquid in the tissue is from the interfascicular or lymph-canalicular system into the lymphatics, and as the fungus is found in the latter as well as in the former, it might be expected *à priori* that the fungus, while increasing, would penetrate from the interfascicular channels into the lymphatics. That this is actually the case may be deduced from microscopic examination; for it can

be seen in many places that the mycelium extends from the lymph-canalicular system into the neighbouring lymphatic vessels in which their ends are provided with the conidia.

In fig. 11, in A & B for instance, the mycelium-filaments, from the terminations of which spring the conidia seen in the lymphatic vessel, can be traced for a long distance into the lymph-canalicular system, from which the lymphatic vessel receives its supply. Not only does the fungus extend from the tissue into the lymphatic vessels, but also into some veins; for I have seen several instances where one could trace the mycelium from the interfascicular spaces into the veins, containing filaments in a state of fructification similar to that observed in lymphatic vessels (fig. 10, V.). The mycelium and its conidia-bearing parts are most easily seen, and with the greatest distinctness, in the distended lymphatics, where they are found in immense numbers, and sometimes form a very dense thallus. They are, however, seen to extend all through the tissue of the corium—in the earlier stages, as mentioned above, only in the deeper stratum of the corium; later also in the superficial stratum of the corium and the subcutaneous tissue. In the latter the fructification is seen in the later stages to go on with such rapidity that the distended interfascicular lymph-spaces at some parts become filled with a zooglœa-like mass, in which here and there the minute spores, the products of division of the spores previously mentioned, can be still recognized to form necklace-like chains.

In Plate 32. fig. 13 this zooglœa is represented in lymphatic vessels and in the interfascicular spaces of the subcutaneous tissue.

We return now to the structural changes in the skin of the pock. In pocks which have been cut out two to three days after their appearance, the rete Malpighii is seen to be many times thicker than in the normal parts, and thickest in the centre. The cells of the deeper and middle strata are enlarged and sharply outlined. Many of them are in the condition of multiplication, as may be deduced from the fact that they possess two nuclei, and that, particularly in the deepest layer, the nuclei are much closer to each other, *i. e.* more numerous. In the flattened cells of the more superficial strata there lie close to the poles of the oblong nucleus highly refractive granules, which are largest and most numerous in the most superficial cells, and become smaller and scarcer in the deeper strata; at the same time the nuclei of many cells of the superficial strata look as if they were vacuolated, viz. sharply outlined with perfectly clear contents.

At the central parts of the pock the rete Malpighii shows other important changes: isolated epithelial cells, or, as is oftener the case, small groups of two, four, or a greater number, are met with, which differ from the rest in containing much coarser granules and possessing very distinct sharp outlines; they are at the same time always more or less rounded: in some of them the nucleus is double or is in the act of division; these occur chiefly in the middle strata of the rete Malpighii. Amongst them some are seen to be darker and more homogeneous than the rest, so that their nuclei are hardly or not at all visible; while others appear to have become confluent, so as to give rise to the appearance of dark more or less homogeneous horny streaks of longer or

shorter dimensions in the section. This transformation of the epithelial elements is at first limited to the central parts of the pock and the middle strata of the rete Malpighii; but as the pock increases in size, the horny streaks, at first isolated, increase in number and length until they become confluent, so as to form one more or less continuous horny stratum, thus dividing the rete Malpighii of the central part of the pock into a deep layer below the horny stratum, and a superficial layer between the newly formed horny stratum and the original stratum corneum. This change goes hand in hand with the growth of the pock in breadth and with the appearance of the central pale depression mentioned on several occasions (see Plate 29. fig. 4 and Plate 30. figs. 5 & 6).

Simultaneously with the formation of the horny stratum, the superficial as well as the deep part of the rete Malpighii (fig. 6, B & C) undergoes remarkable changes. The cells of the former become more transparent and flatter; commencing from the centre of the pock, they gradually assume the characters of horny scales, the nuclei of which gradually disappear; in this way the horny stratum (D in fig. 6) increases rapidly in thickness towards the surface. The cells of the superficial layers of the deep stratum of the rete Malpighii (viz. the layers directly beneath the horny stratum) exhibit rows of highly refractive granules, which are seen to be the more deeply stained by hæmatoxylin the further the cells containing them are from the corium, while the cells themselves are larger the nearer they are to the horny stratum. Many of those nearest to the horny stratum look as if they possessed a thick membrane enclosing clear contents, in which there are here and there a few granules besides the nucleus. These cells are no doubt swollen dropsical epithelial cells.

The deepest stratum of the rete Malpighii is in a state of very active proliferation. This is evinced by the fact that the interpapillary processes grow to extremely large dimensions, and that cylindrical processes composed of young epithelial cells penetrate, *i. e.* grow, into the papillæ of the corium from them, the papillæ themselves being longer and thicker than natural. These epithelial processes penetrate into the papillæ in different depths and in different directions; so that in sections many of them present themselves as isolated patches, surrounded by papillary substance, in the neighbourhood of the Malpighian layer. These, as well as the processes which in the section are not severed from their natural connexion with the rete Malpighii, are composed of the same granular substance as the deepest layer of the rete Malpighii, and contain spherical nuclei at more or less regular intervals, which exactly resemble those of the epithelial cells of the rete Malpighii.

In most cases these processes show in section a distinct division into "territories," each surrounding an individual nucleus. Sometimes, however, this separation is indistinctly seen, and then the areas in the section which correspond to the tips of processes cut through resemble, to a certain extent, large multinuclear giant cells. Considering what has been stated on this subject by certain writers, it is necessary to repeat that the conical, cylindrical, thin or thick, short or long processes which penetrate into the papillæ are direct outgrowths of the rete Malpighii. And it must be added that the

proliferation of the deepest layer of cells of the rete Malpighii can be traced into the depth at every place with great certainty (see fig. 6). In consequence of the extremely active proliferation of the deep stratum of the rete Malpighii at the centre of the pock into the corium, the epidermis as a whole is thicker in the centre than at the periphery, notwithstanding that a great part of it has been converted into a horny mass, which occupies less space than it did before its transformation.

It is further necessary to state that the conversion of the middle layers of the original rete Malpighii into a horny stratum gradually extends outward, *i. e.* towards the periphery of the pock, and that the proliferation of the deep stratum is the more active the more rapidly the horny stratum increases in breadth and thickness.

These observations enable us to see how it happens that the central part of the pock becomes depressed and pale as regards the peripheral portion,—depressed, because a great number of layers of the original rete Malpighii have become converted into layers of horny scales, while the deep stratum of the rete Malpighii grows very actively into the corium; and pale, because the central portion is covered with a thick horny dry membrane, *i. e.* the layers of the rete Malpighii, which have changed in the above-named manner. Consequently, as has been already hinted, the appearance of the depressed pale centre of most of the examples of sheep-pox has nothing whatever to do either with glands or hair-follicles, or with the spreading of the pustules towards the periphery of the pock, for all those changes take place before there is a trace of the formation of the pustules. It is to be noted that the formation of the horny stratum as described above is not constant; for in some pocks it does not occur until after the appearance of the pustules, the superficial layers of the rete becoming gradually transformed into a horny substance, spreading from the stratum corneum towards the depth.

The changes of the other parts of the skin are these:—The whole corium and the whole subcutaneous tissue in the peripheral portion of the pock shows infiltration with lymph-corpuscles; this infiltration is especially marked in the corium around the glands and in the deep subcutaneous tissue. From the peripheral portion the infiltration extends into the corresponding strata of the central parts, but is here very much slighter. The older the pock the more intense is the infiltration of the peripheral part; hence, although the infiltration of the papillary tissue extends a little way in the surrounding zone of normal skin, there is a sharp line of demarcation corresponding to the edge of the pock. In the centre the papillary tissue becomes the more infiltrated the older the pock.

As regards the distribution of the lymph-cells, it is very easy to notice, on those places where the infiltration is not too intense, that most of the lymph-corpuscles are situated around the blood-vessels, and extend from here into the interfascicular channels towards the lymphatic vessels, many of which contain a greater or smaller number of them.

The superficial stratum of the subcutaneous tissue is especially interesting in preparations stained with hæmatoxylin. Here it is seen that the interfascicular lymph-spaces are very much dilated; and one can follow the lymph-cells from around the blood-

vessels of the peripheral part of the pocks with great distinctness into the interfascicular spaces and towards the lymphatic vessels of the central part of the pock; at the same time the interfascicular spaces of this as well as of the deep stratum in the centre of the pock are very well marked by the presence of a homogeneous or slightly granular material, which stains blue in hæmatoxylin, and with which those spaces are more or less filled. This material is in all probability plasma which fills the interfascicular spaces, and which, being alkaline, becomes stained blue by hæmatoxylin.

The connective-tissue corpuscles show marked changes, their nuclei being in the act of division or already divided and the cells themselves swollen and coarsely granular.

Many of the nuclei of the connective-tissue corpuscles appear to be vacuolated.

As regards the glands, we may briefly state that the changes are similar to those of the epidermis. In the hair-follicles and the sebaceous glands the nuclei of the epithelial cells of the most external layer, corresponding to the deepest layer of the rete Malpighii, are in a state of very active proliferation, being smaller and much more numerous than in the healthy parts. The epithelium of the ducts of the sebaceous glands is very much thickened, and the more superficial layers of its epithelium, *i. e.* those nearer to the lumen of the gland, are also composed of cells which are somewhat dropsical, and which contain rows of highly refractive granules near the poles of the nucleus.

The horny transformation of the rete Malpighii also extends to the epithelium of the mouths of the ducts of the sebaceous glands and hair-follicles. The epithelium of the proper secreting part of the sweat-glands seems to resist longer than that of the sebaceous glands and hair-follicles, remaining longer unchanged: pocks, however, which are about six or seven days old and more show the external membrana propria of the sweat-glands thickened; the epithelium which lines the lumen is more or less detached from the muscular coat, and the nuclei of the epithelial cells are in the act of proliferation; in general the epithelial cells become more and more loosened, as well from the muscular coat as from each other.

The nearer the pocks approach the stage of formation of the pustule the more intense becomes the infiltration of the corium, both in the peripheral and central part of the pock. The subcutaneous tissue does not show an increase of the infiltration in its superficial stratum.

The very intense infiltration of the peripheral part of the pock next to the surrounding healthy zone is an additional cause, and perhaps one which weighs materially, why the peripheral part of the pock appears very much elevated in respect to the centre.

A very peculiar change takes place in the lymph-corpuscles which occupy the interfascicular lymph-spaces of the deep stratum of the corium and of the superficial stratum of the subcutaneous tissue, viz. the lymph-corpuscles, or rather their nuclei, break up into a number of small particles, deeply stained by hæmatoxylin; these particles are found of all sizes, from the size of a minute granule up to the size of an intact nucleus of a lymph-corpuscle.

The connective-tissue bundles of the infiltrated corium lose their distinct fibrillar

appearance as the process advances, becoming at the same time much thinner. In advanced pocks, *i. e.* shortly before and during the formation of the pustules, they become homogeneous or finely granular; this is to be found in the papillæ as well as in the deep stratum of the corium and in the subcutaneous tissue (Plate 30. fig. 5).

In all primary pocks which have depressed centres and peripheral thickening the infiltration with lymph-corpuscles presents the characters described in the foregoing pages. In the absence of peripheral thickening the peripheral infiltration was not much greater than the central.

D. *Pustular Stage of the Development of the Primary Pustules.*

Pustulation commences by the formation of isolated vesicles in the rete Malpighii, which, as they gradually increase in number and size, become eventually fused together so as to form larger cavities and canals. This occurs generally at a time when the rete Malpighii has increased so much in thickness that it sometimes exceeds 2 millims. and more in vertical diameter; the papillæ of the corium have reached an extreme length by the extensive growth of the interpapillary process of the rete Malpighii, and at the same time the papillary tissue contains more or less numerous lymph-corpuscles. We have mentioned previously that the infiltration of the papillary tissue is most intense in the peripheral parts of the pock, and that the epithelial cells of the rete Malpighii in the middle layers are very transparent and large, the peripheral substance (membrane) of each cell looking as if it were much thickened. To this it is to be added that the papillary tissue, and in general the superficial stratum of the corium, contains, in its dilated interfascicular lymph-spaces and lymphatics, either a distinct mycelium with the spores as products of its fructification, or those zoogloea-like masses of spherical *Micrococci* or their necklace-like chains. In those primary pocks which show a central depression the formation of the vesicles invariably commences at the periphery and soon extends towards the centre in a horizontal direction, so that the formation of the vesicles in the centre of the pock takes place later than that in the periphery. Notwithstanding this, however, numerous vesicles are seen to make their appearance in the centre, which stand in no direct connexion with those of the periphery. But in pocks of which the central portion, instead of being depressed, is elevated (as in many pocks on the mammary glands), the formation of the vesicles commences centrally and gradually spreads towards the periphery. Now the question arises, In what way do the vesicles form? That the vesicles in their first stage are filled with a transparent fluid lymph and that they afterwards gradually become filled with lymph or pus-corpuscles are well-known facts; but the question of their mode of origin has, I think, not been investigated with sufficient detail. The assertion of LUGINBÜHL that the formation of the pustule is to a great extent due to the appearance of giant cells, must be at once abandoned so far as relates to variola of sheep, for in no instance have I been able to see any indication of such giant cells in the rete Malpighii.

Considering that the infiltration of the corium with lymph-cells, fungi, and serum

(the distension of the interfascicular lymph-system) spreads from the deeper strata gradually towards the surface of the corium, and that the nearer the stage of the formation of the pustules is reached the more the epithelial cells of the middle layer of the rete Malpighii become dropsical and the proliferation of the deeper cells increases in activity, it appears evident that the excessive irrigation of the corium gradually extends towards the surface. I am inclined to think that this is probably attributable to stagnation in the large subcutaneous lymphatics and in the veins; for I have found the lymphatics leading from infiltrated parts dilated and plugged up by dense mycelium and lymph-corpuscles. I have also observed veins densely packed with lymph-corpuscles and mycelium.

It can therefore be easily understood that inasmuch as the lymphatics and veins of the deeper parts of the pocks become prevented from carrying away the morbid products, and inasmuch as there is a constant addition of them, as shown by the increase of the infiltration, it must naturally lead to a stagnation in the passages leading to the efferent vessels, which stagnation may be the cause of the morbid material of these passages being gradually carried in another direction, *i. e.* towards the surface. This view, as we shall see presently, is further supported by the fact that the lymph-corpuscles of the papillary tissue not only find their way in great numbers into the rete Malpighii, but also into the epithelium of the sebaceous glands, the hair-follicles, and the sweat-glands, which structures become surrounded by more and more numerous layers of these bodies. That the spontaneous movement of the lymph-corpuscles is to a certain extent of importance in determining their migration, cannot of course be denied; but it is improbable that this is the only factor, for it is very difficult to see how that could be the case, considering how densely they are packed in some places.

The formation of the vesicular cavities invariably depends on the transformation of individual epithelial cells of the middle layer of the rete (sometimes nearer, sometimes further from the surface) into spherical or elliptical vesicular structures, which possess a thick membrane and clear contents. The process of transformation is usually as follows:—An individual cell expanded by dropsical swelling presses on the surrounding cells so much that they gradually become flattened and so compressed that they almost coalesce. In this way a vesicle is formed, the membrane of which is composed of concentrically arranged scales. These scales when seen in profile appear to be spindle-shaped. Of the epithelial cell from which the vesicular structure originated, all that remains is the nucleus surrounded by a thin zone of granular protoplasm (the original substance of the cell). This remains attached to one side of the vesicle, and finally disappears.

But there is also another way by which individual epithelial cells become transformed into vesicles, *i. e.* by vacuolation. A cell first shows a small vacuole; by the enlargement of the vacuole the nucleus becomes pressed at the periphery, while the original cell-substance expands into a vesicular membrane enclosing the vacuole; with this membrane the nucleus, which becomes more flattened the larger the vacuole grows, is

incorporated. By the gradual coalescence of groups of vesicles, smaller or larger spherical, irregular, or elongated sinuses are formed, which may acquire a large size by the disappearance of the intervening septa composed of compressed epithelial cells. If the formation of the vesicles extends in a horizontal direction and the vesicles are pretty close, the epithelial cells subjacent to them form continuous layers of horny scales, the nuclei of which soon disappear. And this is the case whatever may be the development of the horny stratum, and even when it is not distinguishable. The smaller vesicles contain clear fluid; in the larger there are structures, consisting partly of lymph-corpuscles but principally of mycelium, which may or may not be in fructification. I have preparations in which spores of exactly the kind described in the former chapter, and represented in Plate 32. fig. 11 and Plate 31. fig. 10, V., could be very distinctly traced from the papillæ through the deeper strata of the rete Malpighii into the vesicles.

In some cavities the mycelium is dense and composed of filaments so thin that it looks like a zooglœa, especially where the filaments are beset with very small conidia. In such cavities the fructification of the mycelium is probably going on with very great rapidity and intensity (Plate 29. fig. 18 and Plate 30. fig. 19). In those cavities which lie deepest, that is, most remote from the layer which is the seat of the horny transformation of the rete Malpighii, it can be made out, by the examination of different cavities lying side by side, that by fructification the mycelium may assume an appearance similar to that of zooglœa of *Micrococci*. The comparison of the two conditions can even be made in one and the same cavity, which may contain in one part very distinct mycelium with conidia, in another material like zooglœa—the transition from the former to the latter consisting in this, that the filaments of the mycelium gradually become thinner and their network denser, while the spores diminish in size by division and are more closely aggregated.

The infiltration of the vesicular cavities with lymph-corpuscles from the papillæ takes place in some cases simultaneously with this formation, sometimes later. It commences at the periphery of the pock, where the subjacent tissue is most intensely infiltrated, and spreads from thence towards the centre (*cf.* Plate 32. figs. 14 & 15). Besides the lymph-corpuscles migrating through the deep stratum of the rete Malpighii, there are seen also other small highly refractive bodies which, from their aspect, I am inclined to take as spores.

Many of the lymph-corpuscles themselves contain a number of spherical bodies which, from their characters, cannot easily be assumed to be their nuclei, being of a greenish colour, and being similar to those found free beside the lymph-corpuscles on their way through the deep stratum of the rete Malpighii and in the papillary tissue; they correspond probably also to spores.

This is in accordance with what was found in the pus-corpuscles of fresh lymph (see Plate 31. fig. 10, III., and Plate 29. fig. 3, 1). I can easily imagine that lymph-corpuscles, while migrating from the papillary tissue into the rete Malpighii, take up the spores lying in the former and carry them with them just as they would take up

pigment-granules on a warm stage and creep away with them. As the rete Malpighii becomes infiltrated with lymph-corpuscles, they appear to be eliminated from the corium; for I have preparations of pocks in which, while the very abundant cavities in the rete Malpighii are crammed with lymph-corpuscles, the papillary tissue has become almost barren of them.

In one instance I have had opportunity of observing a primary pock in which an enormous infiltration of the corium with lymph-corpuscles, and the formation of very numerous pustular cavities containing them, had taken place at a relatively very early period, the fourth or fifth day after its appearance. In this case the deepest stratum of the rete Malpighii at the periphery of the pock was actually completely broken through by the contents of the papillæ, whereby a broad direct passage was established between the latter and the intercommunicating pustular cavities. In primary pocks of old standing, when the formation of the vesicles and their infiltration with lymph-corpuscles has reached a very high degree, the layers of the rete Malpighii containing the vesicles nearest the surface are seen to become very much loosened from the subjacent strata of the rete and to detach themselves easily. In this stage, when the papillæ contain few lymph-corpuscles, there are found, in the matrix of the papillæ (which is now transparent, finely granular, or homogeneous), more or less sinuous large spaces very close to the rete Malpighii, which contain a clear lymph and occasionally also masses of *Micrococci*. These spaces enlarge into the rete, and may even become continuous with the deepest vesicles.

I have now only to add a few words relating to the other parts of the skin.

Of the glands of the corium the sebaceous glands deserve the most attention. The epithelium of the glands and their ducts (properly speaking the mouths of the hair-follicles) become immensely enlarged, chiefly on account of their epithelium proliferating so enormously that it is composed, like that of the rete Malpighii, of a very great number of layers. The deepest epithelial cells, the nuclei of which are rapidly dividing and many of them in a state of vacuolation, become smaller and smaller and at the same time more loosely connected with one another.

In the ducts the epithelial cells which are nearer to the lumen are, on the other hand, more swollen, more transparent, and dropsical. The infiltration of the rete Malpighii with pus-corpuscles extends into the mouth of the sebaceous gland and also into the proper secretory parts of the glands. The pus-corpuscles of the surrounding tissue gradually penetrate amongst the epithelium of the former to such an extent that the centre of the gland in some places becomes completely filled with closely packed pus-corpuscles.

Although there is a marked infiltration of the sweat-glands with pus-corpuscles from the surrounding tissue, this infiltration never reaches such an extent as in the sebaceous glands. The epithelium of the sweat-glands becomes more and more loosened under the infiltration, and the lining epithelial membrane becomes broken up into a number of small cells, the nuclei of most of which show vacuolation.

E. *Anatomical Investigation of Secondary Pustules.*

The examination of the secondary pocks, *i. e.* those of the general eruption, proves that the anatomical changes are substantially the same as in the primary. We find also here, at the outset of the process, thickening of the rete Malpighii and œdema of the corium, combined with the presence of lymph-corpuscles around the blood-vessels and extending hence into the distended lymph-canalicular system.

The infiltration of the corium with lymph-corpuscles extends very soon, however, upwards into the papillary stratum and downwards into the subcutaneous tissue. In general it may be said that in the secondary pocks the whole process goes on much more rapidly, *i. e.* the stage of pustulation is much sooner reached than in the primary pocks.

In most of the secondary pocks on the lip, the pustulation was seen to be going on as early as from two to four days after their appearance; in those of the walls of the chest and abdomen the same thing was seen after from three to seven days.

The infiltration of the subcutaneous tissue and corium was always found to be greater in the peripheral part than in the central. This was better marked in those pocks which were of long standing, *i. e.* which developed slowly, and particularly in those in which a central depressed and a peripheral thickened part could be distinguished. In the rete Malpighii the same immense overgrowth of the interpapillary processes occurred as in the primary pocks, and the cells of the middle layers showed the same tendency to become soon dropsical. I have not observed the formation of the horny stratum; but in the central parts of many of the pocks I have noted the conversion of groups of epithelial cells into horny masses.

As regards the interfascicular lymph-channels and the lymphatic vessels of the corium and their contents, I have only to repeat what I have stated as regards the primary pocks, viz. that one is able to follow the at first zooglœa-like masses of *Micrococci* into necklace-like filaments, which gradually become more and more branched, so as to form a delicate mycelium; in some places the filaments of the mycelium bear conidia, and show the same fructification as those mentioned in the former section. The formation of the vesicles takes place in the same way as in the primary pocks, viz. by dropsical swelling and vacuolation of individual epithelial cells.

The pustulation commences as a rule in the centre, and spreads rapidly into the periphery. The vesicles make their appearance in great numbers simultaneously, and are situated chiefly in the middle layers of the rete Malpighii, but are generally met with much nearer the corium than in the primary pocks. It is worth noticing that when the vesicles lie deep in the rete Malpighii, the expansion of the individual vesicles goes on at the expense of the interpapillary processes; so that as the vesicles enlarge the interpapillary processes become shorter, until the line of demarcation between the rete Malpighii and the corium becomes almost as even as in the normal state: in the latter case, therefore, the deepest cells of the rete Malpighii appear very much compressed, as if the rete had been dragged over the surface of the corium. There is

another point, as regards the distribution of the vesicles, which I think important, viz. that even in those pocks in which there was a very marked central depression, the most numerous and best developed vesicles were found in the centre, and they became smaller and fewer the nearer to the periphery—thus showing clearly that the depression in the centre is not caused by the disappearance of previously existing vesicles.

I have now to call the attention of the reader to Plate 29. fig. 16, in which the formation of the vesicles is shown, as well as the presence of an *Oidium*-like fungus in their contents.

In some vesicles the mycelium, which here also is composed of filaments of very various thickness, is imbedded in a finely granular matrix, which I suppose is only coagulated plasma; in others the matrix is almost homogeneous, and is stained slightly with carmine and hæmatoxylin as in D. After some time pus-corpuscles are seen to penetrate from the papillary tissue through the deeper strata of the rete Malpighii into the vesicles, just as in the primary pocks. So also the transformation of the mycelium in the vesicles by rapid fructification into a zoogloœa-like mass of *Micrococcus* occurs in the manner already described. I have represented the characters of the mycelium and the spores attached to it in Plate 29. fig. 17, drawn with every possible accuracy from the contents of a vesicle of a secondary pock.

The mycelium, as well as the spores, possess a greenish colour, and are of a bright and shining aspect.

As peculiarities which I had not seen in any of the primary pocks, and in only one secondary pock of the upper and one of the lower lip, may be mentioned the occurrence of blood in a single vesicular cavity, the rest not containing any. This vesicle was situated rather deeply in the rete Malpighii. In another instance I observed the effusion of blood into the sheath of a hair-follicle and into the adventitia of an artery. In both cases the blood was contained in a number of large irregular spaces communicating with each other.

I am unable to refer the fungus of which we have spoken in the foregoing and in this chapter, and which, as we have mentioned and figured, occurs first in the tissue of the corium and its lymphatics and is gradually carried or penetrates into the vesicles formed in the rete Malpighii, to any described species, and would propose to call it provisionally *Oidium variolæ*.

Although nobody could, from the very great distribution of this fungus through the whole pock, take it to be a mere accidental entophyte, yet it might be objected that we are wrong in asserting that its development begins and ends in *Micrococcus*. Against this objection it must be maintained that, besides our being able to follow the one into the other as far as place as well as time is concerned, we also find (and this appears to me to be of still greater importance) at one time only the one organism and at another time only the other; we have found first only the *Micrococcus*, then we have found only the *Oidium* form, which we finally see again replaced by *Micrococcus*. Whether a form of vegetation corresponding to *Oidium variolæ* in sheep is to be met with in

the cow-pock or in human smallpox, is a very important question, and one which deserves alike the attention of physicians and anatomists.

Explanation of the Plates.

PLATE 29.

Fig. 1. Lymph from pustules of sheep-pox, kept in a sealed capillary tube. (Examined March 10, 1874, with Hartnack's ocular 3, objective 9.)

1. Transparent masses of various sizes, containing granules, some of which are small, pale, and indistinct, others large and shining.
2. Transparent spheroid bodies containing necklaces.
3. Highly refractive *Micrococci* in proliferation, forming dumb-bells, *Sarcina*-like structures, and small colonies.
4. The same, between decolorized blood-disks.
5. *Bacterium.*
6. Colonies of *Bacterium termo.*
7. Shining *Micrococci* imbedded in a greenish matrix, some being surrounded by a thin membrane.
8. Groups of bodies similar to those in 2 and 3.

Fig. 2. Similar preparation to fig. 1, but which had been kept for 24 hours in the incubator. (Examined March 11, with Hartnack's oc. 3, obj. 10, immersion.)

1. Network of fine filaments beset with *Micrococci*; transparent spheroids like those in fig. 1, 2.
2. Network of filaments more defined, with spheroid bodies budding from it.
3. Part of the same preparation, kept in the incubator till March 17.

Fig. 3. Lymph from pustule of sheep-pox kept for 24 hours at a temperature of 32° C. (Examined March 25, with Hartnack's oc. 3, obj. 8.)

1. Homogeneous sporids, some free, others contained in pus-corpuscles. The same sporids of a paler colour, containing one or two *Micrococci.*
2. *Micrococci* forming necklaces.
3. The same in groups.
4. The same in colonies connected by filaments of various lengths.
5. Diagrammatic representation of the relation of 1 to sporids and of 2 to *Micrococcus* (*Cryptococcus* of Hallier).

Fig. 4. Preparation from a vertical section of the thickened epithelium of the peripheral part of a pock three days after its first appearance. (Hartnack's oc. 3, obj. 7.)

A. Horny layer.

B. Superficial layers of the rete Malpighii. Near the poles of the nucleus

of some of the cells rows of highly refractive granules may be seen. These granules become fewer and smaller as they approach the middle layers, C, of the rete Malpighii, and eventually disappear. Many of the nuclei of the epithelial cells of the middle layers and of the lower layers, D, are seen to be divided. In D some of these divided nuclei are vacuolated or dropsical. In the middle layers epithelial cells may be seen, both isolated and in groups, which are sharply defined and opaque. These are being converted into a homogeneous horny substance.

PLATE 30.

Fig. 5. Vertical section of half of a pock, excised seven days after its appearance, showing a pale central and a thickened reddish peripheral part. The figure shows the general distribution of the infiltration. (Oc. 3. obj. 2.)

A. Part of the pustule where the peripheral thickening is in contact with healthy tissue.

B. Epidermis of healthy skin.

C. Epidermis of central part of pustule, showing the rete Malpighii divided into three layers—a superficial layer composed of nucleated somewhat flattened cells, a middle, horny layer, and a lower layer composed of polyhedral, nucleated, granular epithelial cells, the true rete Malpighii. (Compare with fig. 6.)

D. Superficial layer of corium, showing infiltration with lymph-cells around the blood-vessels and in the lymphatics.

E. Deep layer of corium, containing sebaceous and sweat-glands.

F. Superficial layer of subcutaneous tissue, showing the interfascicular lymph-channels much distended. The smaller branches of the canalicular system could not be represented, owing to their extreme minuteness.

G. Deep layer of subcutaneous tissue, showing fat-cells and transverse sections of blood-vessels and lymphatics.

It will be observed that infiltration with lymph-cells occurs abundantly at the periphery of the pustule, and extends through all the layers, but chiefly in the corium and deep subcutaneous tissue.

Fig. 6. Vertical section of central depression of a primary pustule seven days old, showing the changes in the epithelium. (Oc. 3, obj. 7.)

A. Horny layer.

B. Superficial layers of rete Malpighii undergoing conversion into horny scales.

C. Deep layers of rete, composed of protoplasmic, germinating epithelial

cells. In the deepest layer are seen conical or cylindrical processes composed of young epithelial cells (E), which penetrate into the papillary tissue of the corium, in which dilated blood-vessels and cellular elements may also be observed.

D. Middle horny stratum connected by pillar-like processes with the interpapillary processes of the deep rete Malpighii. The cells below the middle horny stratum contain series of granules of an arrangement and distribution similar to those represented in fig. 4. Some cells immediately below D are seen to be enlarged, dropsical, and in a state of vacuolation. The interpapillary epithelial processes and the papillæ are much enlarged.

PLATE 31.

Fig. 7. Section through a primary pustule 24 hours old, in which are seen transverse sections of lymphatic vessels (A) of the deep layer of the corium, as well as the interfascicular lymph-channels (B) containing connective-tissue corpuscles. The lymphatics are filled with zooglœa. (Oc. 3, obj. 7.)

Fig. 8. Similar preparation. (Oc. 3, obj. 7.)

A. Lymphatic vessels containing granular material (zooglœa) and a filamentous substance (necklaces of *Micrococci*).

B. Interfascicular lymph-canaliculi, in connexion with the lymphatic vessels, and containing connective-tissue corpuscles. In I. a blood-vessel (C) is seen to penetrate a lymphatic vessel (perivascular lymphatic); in II. a valve D is represented.

Fig. 9. Similar preparation from a pustule about 60 hours old. (Oc. 3, obj. 7.)

A. Lymphatic vessels lined with endothelium, like those in previous figures.

I. contains lymph-corpuscles, zooglœa, and filaments, the last mentioned being in II. much more numerous.

Fig. 10. Sections of lymphatic vessels lined with endothelium. Mycelium is seen to be contained in them. Nos. I. & V. are drawn with HARTNACK'S oc. 3, obj. 8; Nos. II., III., and IV. with oc. 3, obj. 7.

I. Part of subcutaneous lymphatic of a pustule about 60 hours old.

II. & III. Lymphatics of corium of a pustule of the same period.

IV. & V. Preparations from a similar pustule.

PLATE 32.

Fig. 11. Section through lymphatic vessels of the deeper layer of the corium of a primary pustule 30 hours old. (Oc. 3, obj. 11, immersion.)

In both A & B the conidia of the mycelium represented in fig. 10 and the proliferation of the spores may be seen.

Fig. 12. From a section of the subcutaneous tissue of the same pustule as fig. 11 (oc. 3, obj. 8), showing:—

A. The distended interfascicular lymph-spaces.

B. Connective-tissue corpuscles in an altered condition.

C. Bundles of connective tissue.

Fig. 13. Section through subcutaneous tissue of a primary pustule from 3 to 4 days old. (Oc. 3, obj. 7.)

A. Lymphatic vessels.

B. Interfascicular lymph-spaces.

C. Bundles of connective tissue.

The lymphatic vessel and the interfascicular spaces contain *Micrococci* either in the form of zoogloœa or of necklaces.

The lymphatic vessel is lined with endothelium. On the surface of the bundles of connective tissue the corpuscles are seen to extend into the interfascicular spaces.

Fig. 14. Vertical section through the periphery of a primary pustule 10 days old. (Oc. 2, obj. 7.)

A. Horny layer.

B. Rete Malpighii. There is a striking difference in the thickness of the epidermis in the pustule and in the neighbouring healthy skin. In the former the rete Malpighii is divided into three parts by a middle horny layer, viz. a superficial layer and a deep layer which is undergoing active proliferation, sending out long thick interpapillary processes.

C. Superficial layer of corium. It is seen to be very different from that of healthy skin, being infiltrated with lymph-cells and having large papillæ.

D. A vesicle in the rete Malpighii, the lymph-corpuscle of which can be traced to have migrated from the papillæ into the deep layer of the rete. To the left of the drawing small cavities containing lymph-corpuscles may be observed in the deep layer of the rete Malpighii. These are the first traces of the formation of vesicles.

Fig. 15. Vertical section of the rete Malpighii below the middle horny stratum (see figs. 6 & 14) from a primary pustule 12 days old. The epithelial cells are seen to be much enlarged, many of them somewhat dropsical and vacuolated and changed into vesicles of various sizes. They are confluent, and thus form channels. It may be observed that there is a migration of lymph-corpuscles from the lower portion (B) towards the surface (A). The highly refractive bodies contained in the vacuolated epithelial cells originate, probably, partly from the nuclei of those cells, partly from lymph-corpuscles. Some of them are of a greenish colour, and are contained in lymph-corpuscles. A granular

or filamentous substance may be observed in some of the superficial vacuolated epithelial cells, which is probably connected with the fungus found in the pustules (see figs. 18 & 19).

PLATE 29.

Fig. 16. Vertical section of rete Malpighii of a secondary pustule—that is, one forming part of the general eruption. (Oc. 3, obj. 7.) The rete Malpighii is thickened, and in the middle layers single epithelial cells are seen being converted into vesicles.

A. Horny layer.

B. Deep layer of the rete Malpighii. In both layers of the rete many of the nuclei of the epithelial cells are changed into well-defined vesicles.

C. Vesicles containing the mycelium of the *Oidium*-like fungus.

D. Vesicle in which the matrix of the mycelium has been stained with carmine.

Fig. 17. Part of the contents of the pustule represented in fig. 16. (Oc. 3, obj. 10, immersion.) The *Oidium*-like fungus seen in the previous figure is imbedded in a finely granular substance (coagulated plasma).

Figs. 18 (Pl. 29) & 19 (Pl. 30) are both preparations from a primary pustule, showing the identity of the fungus found in primary and secondary pustules. (Oc. 3, obj. 10, immersion.)

[The research to which the present paper relates has been made as one of the series of scientific investigations which the Lords of the Council are pleased to authorize me to have conducted at their expense in aid of Pathology and Medicine.

The paper itself, being of the nature of a Report for the Lords of the Council, may probably appear entirely or in part as a Parliamentary Publication; but the immediate interest of the facts makes me think that the Royal Society will be glad to be at once possessed of them; and I therefore avail myself of their Lordships' permission to communicate the paper to the Society.—John Simon.]

Fig. 1.

Fig. 4.

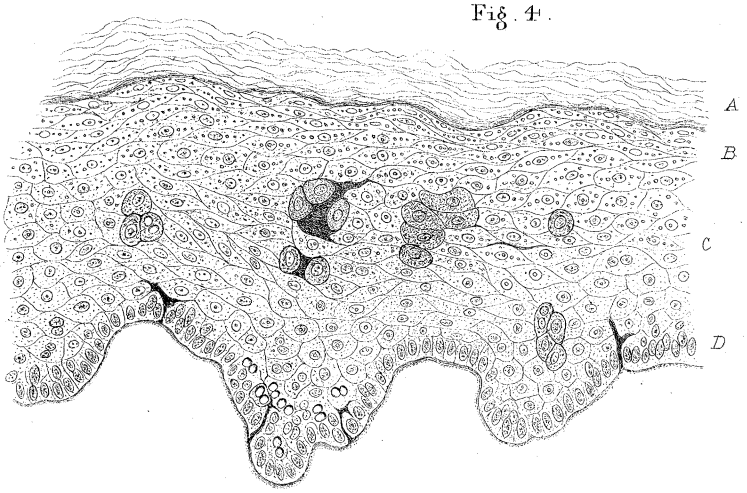


Fig. 3.

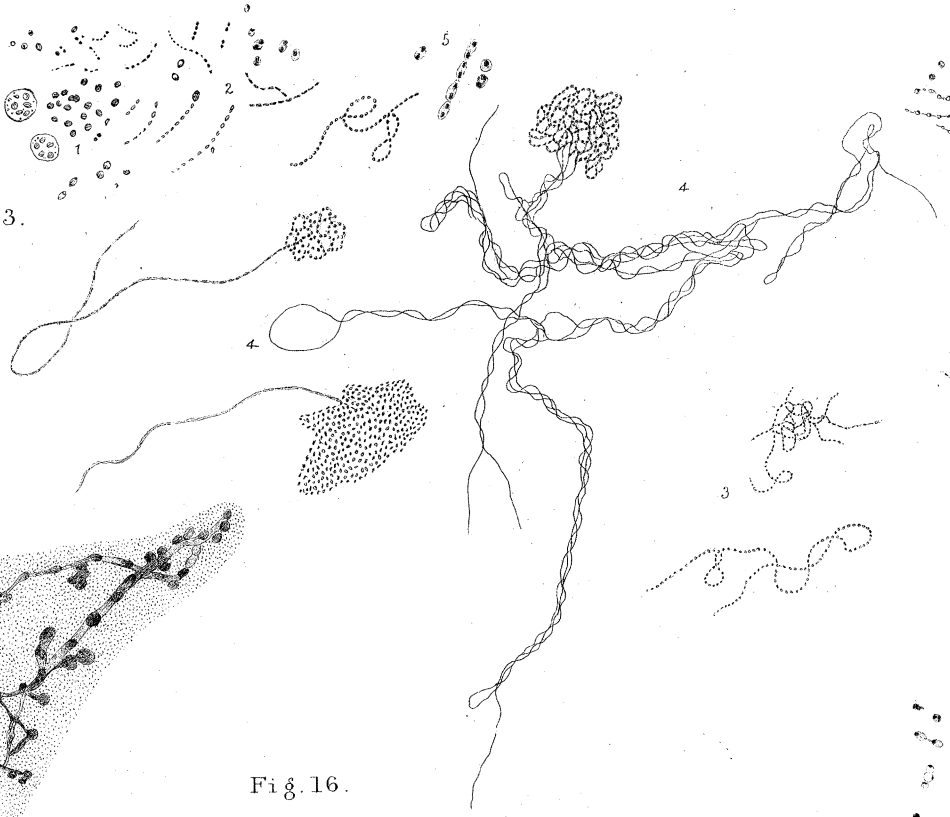


Fig. 2.

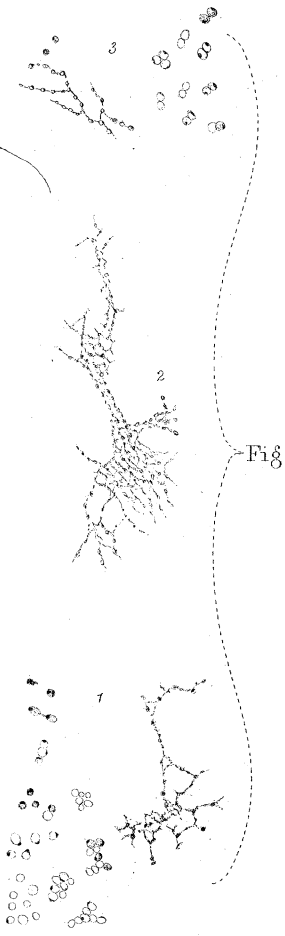
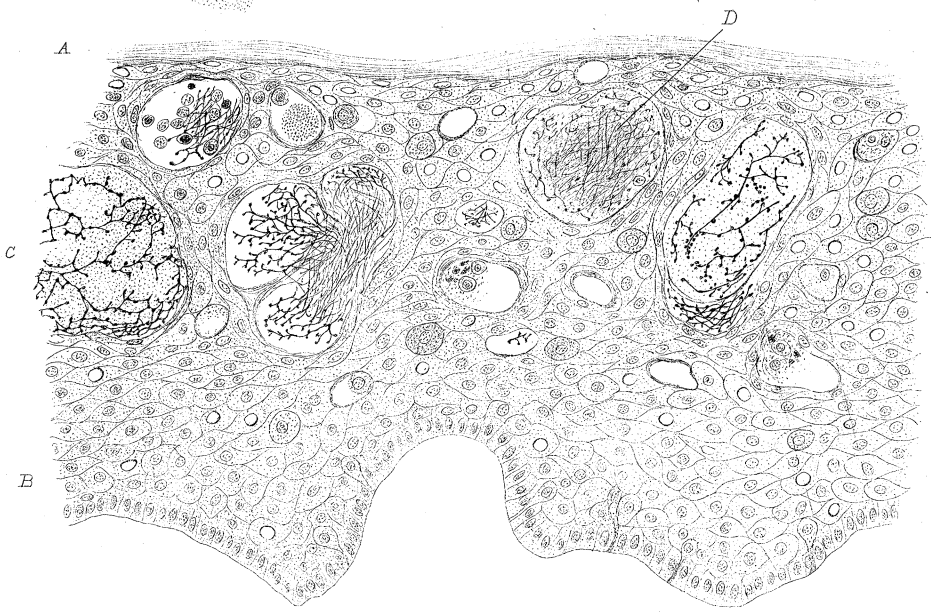


Fig. 17.

Fig. 16.



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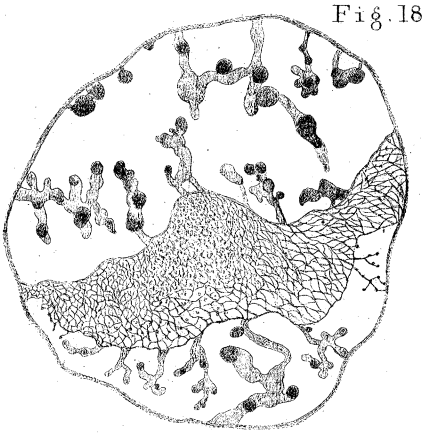
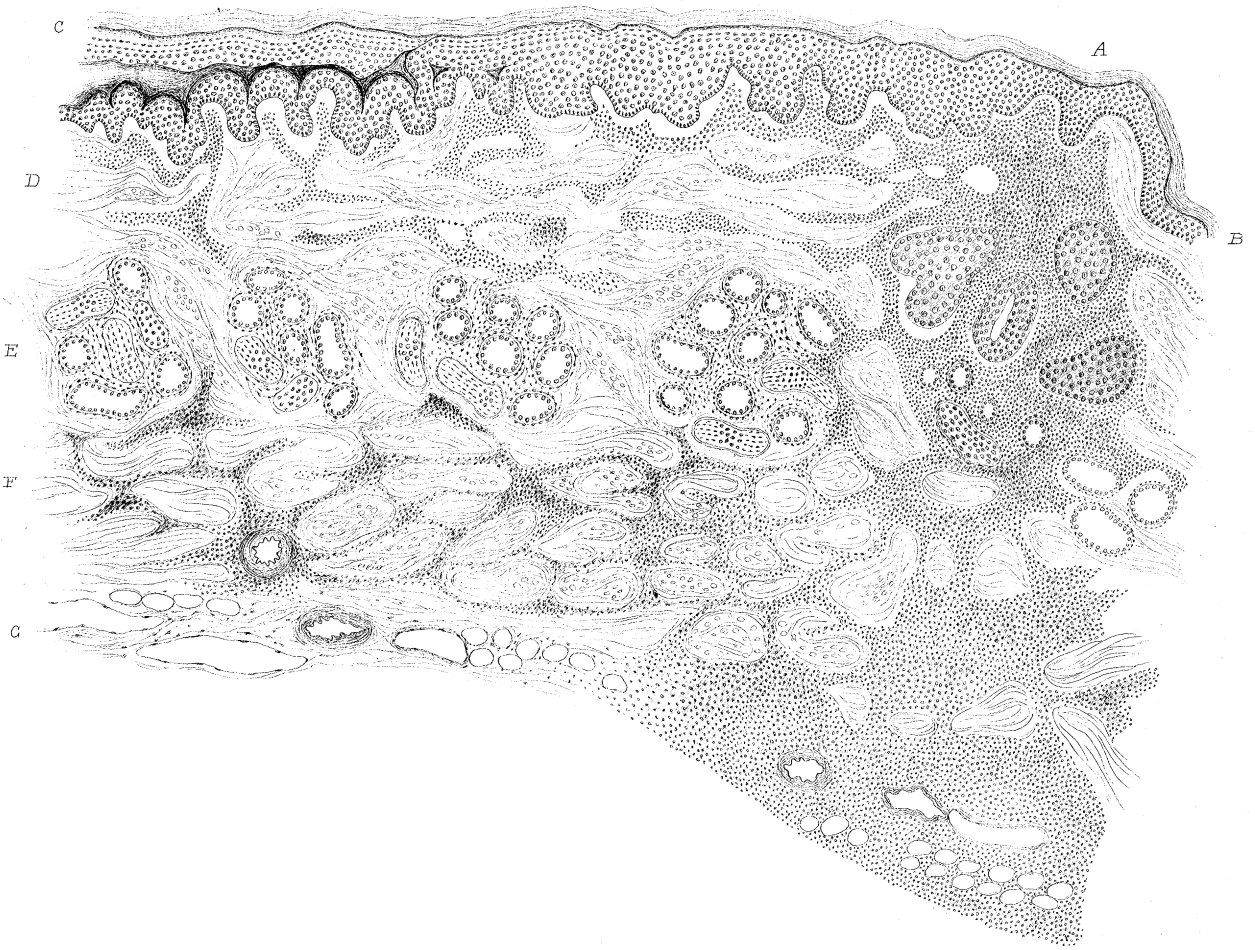


E. Klein del.
W.H. Wesley sc.

W. West & Co. imp.

Fig. 5.



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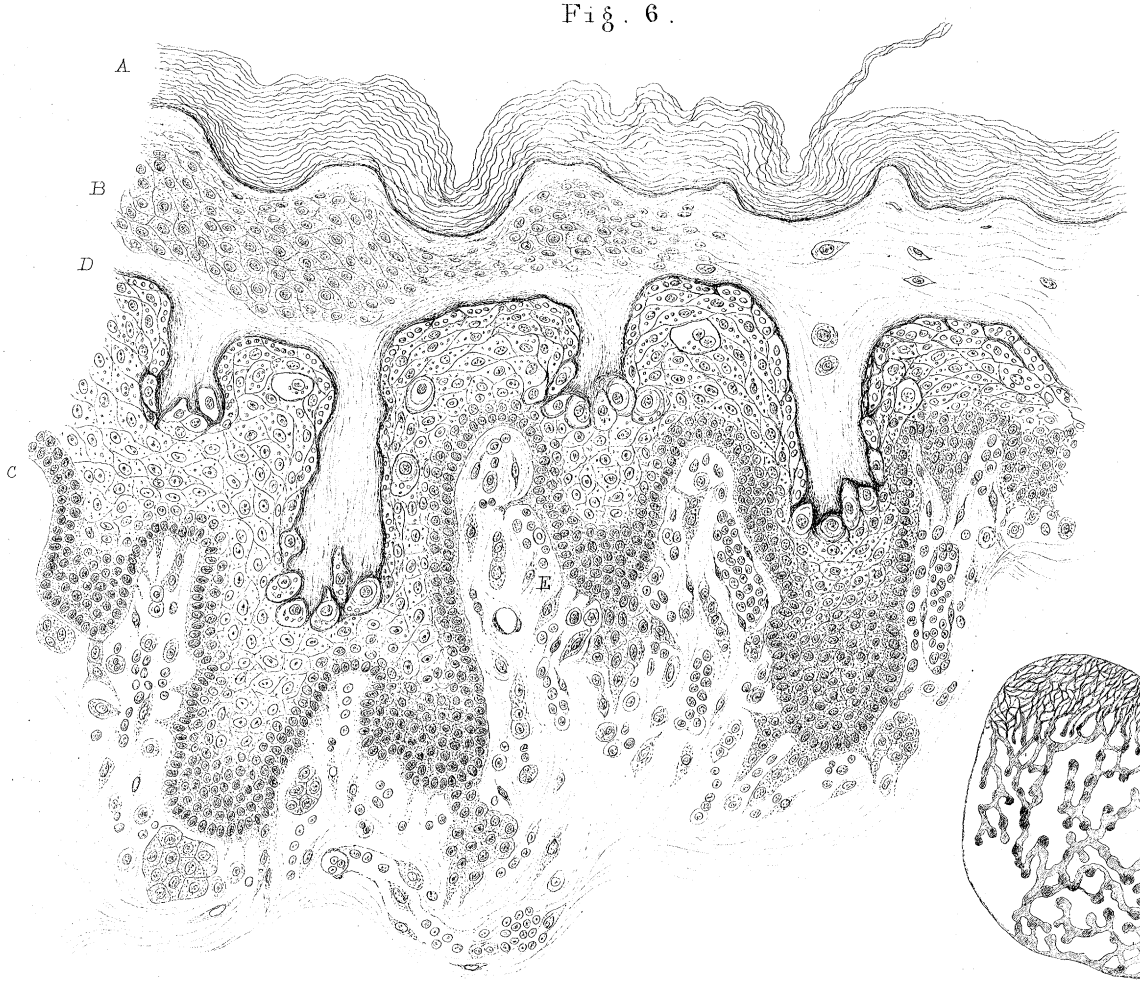

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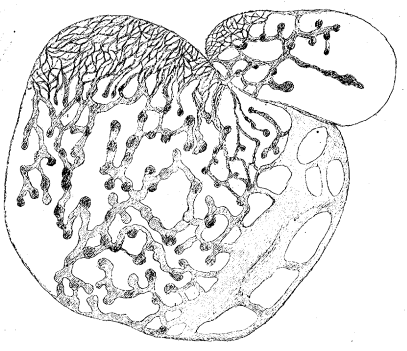


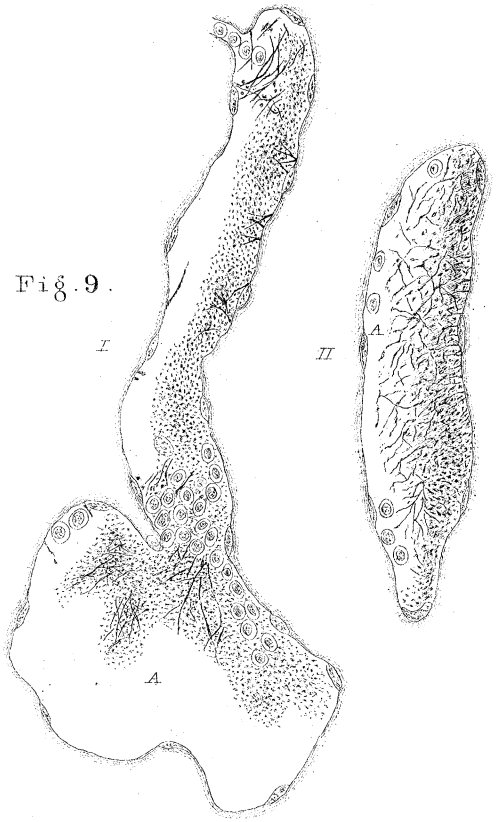
E. Klein del.
W.H. Wesley sc.

W. West & Co. imp.

Fig. 7.

Fig. 8.

Fig. 9.



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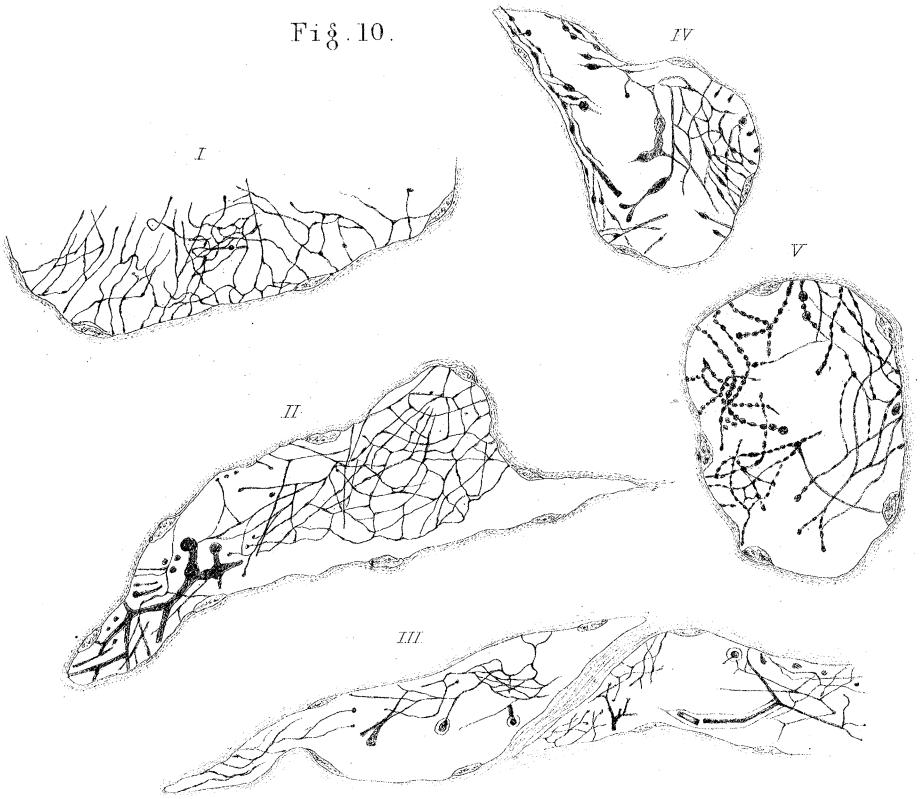
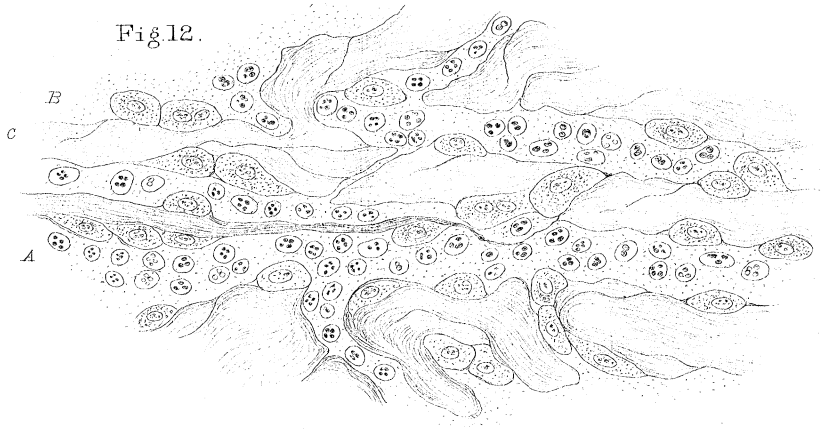
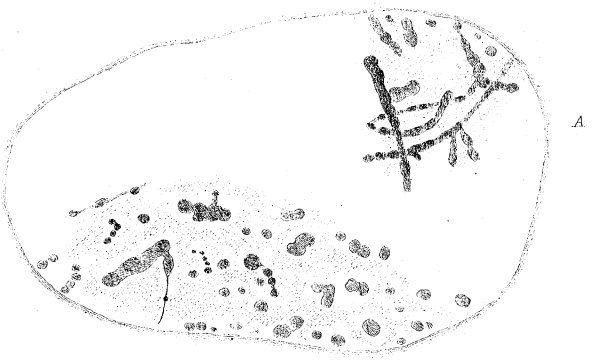


E. Klein del.
W.H. Wesley. Sc.

W. West & Co. imp.

Fig. 12.





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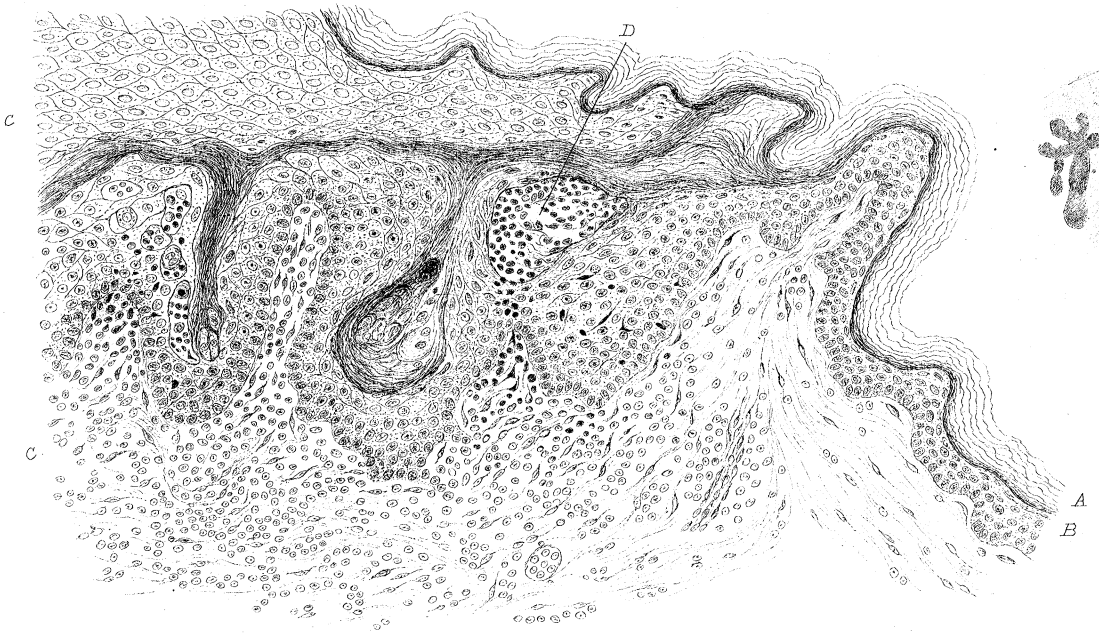

Fig. 11.

Fig. 15

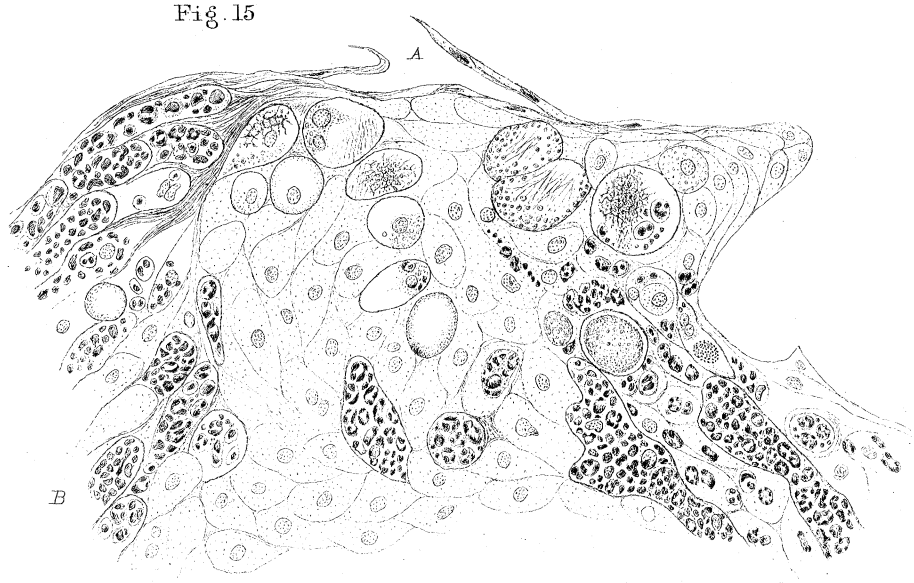

Fig. 13.