

A STUDY OF THE CONTENTS OF THE VESICLES AND PUSTULES OF SMALLPOX.

A PRELIMINARY REPORT.*

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PHILADELPHIA.

Although we have convincing evidence that the *causa causans* of smallpox is contained in the purulent contents of the cutaneous lesions, the elusive micro-organism has thus far defied all efforts to establish its identity. The failure to discover the cause of variola has not been due to any inactivity on the part of investigators, for researches in this field have already given rise to a most extensive literature.

The bacteriologic investigation of smallpox is indissolubly associated with the study of the cause of vaccinia; according to the most authoritative opinion these two diseases have a common origin, and depend in all probability on the same causative agent.

HISTORICAL SUMMARY.

Sacco, in 1809, was the first to point out the presence in vaccine lymph of motile granules. Since this time scores of investigators have noted the presence of cocci of various kinds in vaccine lymph. In addition, larger round and oval bodies, not cultivable on ordinary media, have been described, first by Renault in 1881 and later by Guarnieri and others, and regarded by these observers as animal parasites. That bodies such as have been described exist both in vaccinal and variolous fluid is beyond contention. As in the case of the alleged cancer parasite the question is, are these bodies the result of cytoplasmic changes or are they in reality protozoa? Each side in this controversy has its array of champions, and the battle is being warmly waged.

Guarnieri and his school regard the vaccine and variolous bodies as sporozoa. Ferroni and Massari believe that they are derived from the nuclei of epithelial cells. Salmon states that the staining properties of the so-called parasites correspond in all respects with those exhibited by the nuclei of migratory leucocytes, and concludes after careful study that the pseudo-parasite is derived from this source. Ewing has recently declared that at least some of the vaccine corpuscles in human vascular tissues have their origin in degenerated and fragmented red blood cells. Whether or not these bodies bear any etiologic relationship to vaccinia and variola time and further study alone will determine.

It is pretty generally admitted that the micro-organisms in the fluid of vaccine and variolous lesions which are readily cultivable on ordinary media play no part in the production of these diseases. Klein and Copeman have independently found in vaccine and smallpox lesions a minute bacillus, which the latter observer has been able to grow on hens' eggs. Copeman's inoculation experiments with this organism are highly interesting, but as he himself admits they are as yet scarcely conclusive.

The preliminary work herewith presented is confined to a morphologic and cultural study of the contents of variolous vesicles and pustules.

THE TECHNIC OF THE PRESENT RESEARCH.

Cultures were made from 82 lesions in 51 cases of smallpox. The technic employed was as follows: The

skin was washed with 95 per cent. alcohol and wiped dry with sterilized cotton; the vesicle or pustule was then transfixed with a two-edged lancet or a double-edged needle and one-half of the epidermal roof lifted up. A platinum loop was then inserted and a drop of the contents transferred to the culture tube. The earliest lesion cultured was a third-day vesicle. The investigator, who is familiar with smallpox and who has tried to obtain material for culture, knows how difficult it is in the early lesions to secure sufficient fluid to deposit on nutrient media. In unmodified smallpox with slow evolution of the efflorescence it is frequently impossible as late as the fourth day of the eruption to obtain a satisfactory amount of variolous lymph for culture purposes. On the other hand, in smallpox modified by vaccination one may at times secure pus from the lesions on the third day of the eruption. The writer was careful in cultivating early lesions to see that the platinum loop contained fluid and that this was properly placed on the media used for cultivation. The number of tubes inoculated was considerably in excess of 82, inasmuch as cultures were not infrequently made on three different media from the same pustule. The media employed were nutrient agar, glycerin agar, glucose agar, bouillon, Loeffler's blood serum and coagulated egg albumin. The majority of the cultures were made on plain agar alone, but the other media were also freely employed. The tubes were incubated at a temperature of 37.5 C.

THE RESULTS.

Of the 82 lesions cultured 64 gave absolutely negative results, the culture tubes remaining perfectly free of growth of any kind. The remaining 18 tubes exhibited cultures presently to be described.

The age of the lesions from which cultures were taken is appended in tabular form. The day mentioned is the day of the eruption and not of the disease.

Third day	1 culture,	1 negative,	0 positive.
Fourth day	10 cultures,	10 negative,	0 positive.
Fifth day	12 cultures,	11 negative,	1 positive.
Sixth day	12 cultures,	12 negative,	0 positive.
Seventh day	13 cultures,	10 negative,	3 positive.
Eighth day	7 cultures,	5 negative,	2 positive.
Ninth day	13 cultures,	9 negative,	4 positive.
Tenth day	7 cultures,	4 negative,	3 positive.
Eleventh day	4 cultures,	2 negative,	2 positive.
Twelfth day	2 cultures,	0 negative,	2 positive.
Thirteenth day	1 culture,	0 negative,	1 positive.

Total 82 cultures, 64 negative, 18 positive.

Of cultures made from lesions before the seventh day of the eruption 33 out of 34 proved to be sterile.

In 10 cultures the *Streptococcus pyogenes* alone was found.

In 2 cultures it was associated with a small bacillus resembling the diphtheria organism.

In 3 cases a small bacillus resembling the diphtheria bacillus was found.

In 2 cases the *Staphylococcus pyogenes aureus* was found alone, and in one more case associated with the streptococcus. On several occasions a growth was obtained from a pustule on one medium; for instance, bouillon, and negative cultures obtained on agar and blood serum. These cases have been classed with the positive cultures.

COMPARISON WITH THE WORK OF EWING AND BERGEY.

These results are in striking contrast with those recently obtained by Dr. James Ewing of New York, and embodied by him in a paper before the Association of American Physicians. He made cultures of the skin lesions in 23 cases of smallpox. In 17 he obtained the streptococcus in pure culture, in one case streptococcus

* Read at the Fifty-third Annual Meeting of the American Medical Association, in the Section on Pathology and Physiology, and approved for publication by the Executive Committee: Drs. A. Stengel, Winfield S. Hall and Frank B. Wynn.

and *Staphylococcus pyogenes aureus*, in one streptococcus and a bacillus (Martin's bacillus?), and in a late lesion *Staphylococcus pyogenes aureus*. In one of the sterile cases streptococci were seen in sections of the skin. In other words, streptococci were found in 87 per cent. of the 23 cases examined. In my investigation, 77 per cent. of the cultures of 82 lesions remained sterile. I am at a complete loss to reconcile this discrepancy. I have in many cases deposited on the culture medium a large drop of yellowish creamy pus from a sixth, seventh or eighth day pustule and have failed to secure any growth from it. At the end of twenty-four or forty-eight hours the yellowish pus could still be seen, but no colonies were visible.

In view of the wide divergence in the findings obtained by Dr. Ewing and myself I have recently communicated with Dr. D. H. Bergey, first assistant in the laboratory of Hygiene of the University of Pennsylvania, to whom I had some months previously sent variolous lymph for certain investigations which he desired to carry out. This material was collected and sent in sealed sterilized capillary tubes. Only the largest pustules were selected in order to facilitate the gathering of the lymph, con-

Roger in his work on "*Les Maladies Infectieuses*," page 456, says:

Frequently we have examined and cultured pus from variolous lesions without discovering any organism colorable or cultivable by present-day methods.

In well-marked cases of smallpox there frequently develops during the period of incrustation, about the twelfth to the fourteenth day, a number of flat blebs, a condition which may be appropriately called an impetigo variolosa. These blebs may result from direct conversion of variolous pustules into blisters, the lesions enlarging peripherally and the contents becoming thinned; or a reddish ring with raising up of the epidermis may form around the pustules and grow by peripheral extension. More infrequently blebs may form on healthy inter-pustular areas of skin. Under all these circumstances the affected pustules fill up with a watery dirty-yellow fluid which swarms with streptococci, diplococci and at times also bacilli. The fluid from a series of such lesions was inoculated on culture media. In every instance a growth was obtained in which streptococci were found in abundance. A small quantity of fluid from a large bleb was injected subcutaneously into the abdominal wall of a

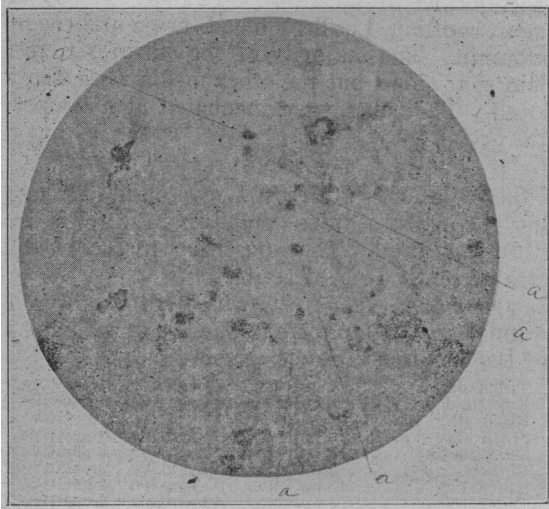


Fig. 1.—From fourth day smallpox vesicle. In Figures 1, 2, 3 and 4, "a" represents the so-called vaccine or variolous bodies. Each illustration is magnified 670 diameters.

sequently the fluid represented late lesions, certainly not earlier than seventh-day pustules.

Dr. Bergey has kindly permitted me to incorporate the results of his work in this paper. I quote that portion of his letter referring to the cultures:

The findings in each of the 12 cases examined are herewith given in detail:

CASE 1.—*Streptococcus pyogenes*, *Staphylococcus pyogenes aureus*, *Bacillus pseudo-diphtheria*.

CASE 2.—*Streptococcus pyogenes*, *Staphylococcus pyogenes aureus*.

CASE 3.—Cultures remained sterile.

CASE 4.—*Streptococcus pyogenes*, *Staphylococcus pyogenes aureus*, *Bacillus pseudo-diphtheria*.

CASE 5.—*Streptococcus pyogenes*, *Bacillus pseudo-diphtheria*.

CASES 6 and 7.—Cultures remained sterile.

CASE 8.—*Streptococcus pyogenes*.

CASES 9, 10, 11 and 12.—Cultures remained sterile.

It is seen from the above that of the 12 specimens of lymph examined 7 remained sterile. This would indicate a negative result in 58 per cent. of the examinations made. If the percentage of negative results in the seventh day and older lesions in my own series is computed it will be found to be 64 per cent.

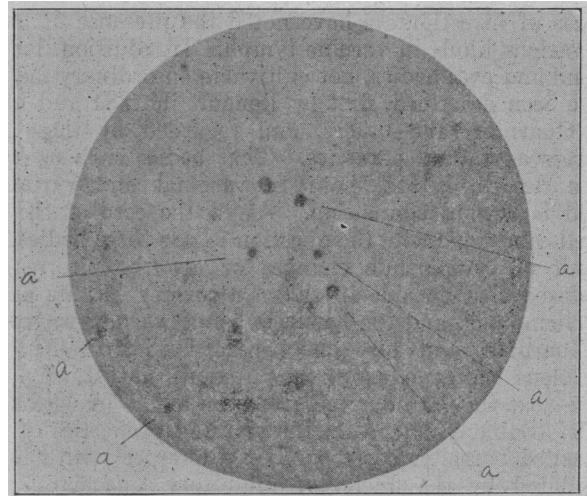


Fig. 2.—From fifth day vaccination.

dog. In a few days there developed an egg-sized swelling apparently filled with a fluid material. A small bleb appeared on the surface of this swelling and rupturing left a dime-sized superficial ulceration. The tumefaction, however, subsided without breaking down, and the animal exhibited no further evidence of local or general disturbance.

MICROSCOPIC EXAMINATIONS OF VARIOLOUS FLUID.

Smears of 15 vesicles and pustules were made and subjected to microscopic study. Lesions of different age from the fourth to the twelfth days were in this manner examined. Two smears of each lesion were prepared, the one being stained with Loeffler's methylene blue and the other with thionin. In addition some were stained with eosin-methylene-blue and with Gram's stain. It would occupy too much space to go into a detailed description of each slide. All of the preparations contained a considerable number of polymorphonuclear leucocytes and also mononuclear leucocytes. These were, of course, more abundant in the fully developed pustules. Eosinophiles were met with only in a few preparations. Nearly all the preparations showed more or less leucocytic degeneration. A number of large granular leucocytes without colorable nuclei were noted. Shreds of fibrin

were abundantly present in nearly all of the specimens. Bacteria were conspicuous by their absence. In some of the later lesions a few micrococci singly and in pairs were found on careful examination. On one or two occasions a few chains of short streptococci could also be discovered. The paucity of micro-organisms in the thick viscid pus was at first a source of considerable surprise. When cultures were made at a later date the explanation of this became evident.

Whenever the material in the pustules began to be converted into a watery substance; in other words, when pustulo-blebs began to form, the microscopic picture was at once changed. This fluid under the microscope was seen to swarm with myriads of streptococci, micrococci in pairs and not infrequently bacilli. This observation is of interest in view of the work of Sabouraud of Paris, who claims the streptococcus to be the cause of "impetigo contagiosa." The development of an extensive impetigo in the course of smallpox, by no means a rare complication, prolongs the convalescence and increases the liability to boils, abscesses, erysipelas and cutaneous gangrene. In a few cases the writer has seen death result directly from this extensive secondary involvement of the skin. Pus from a number of abscesses,

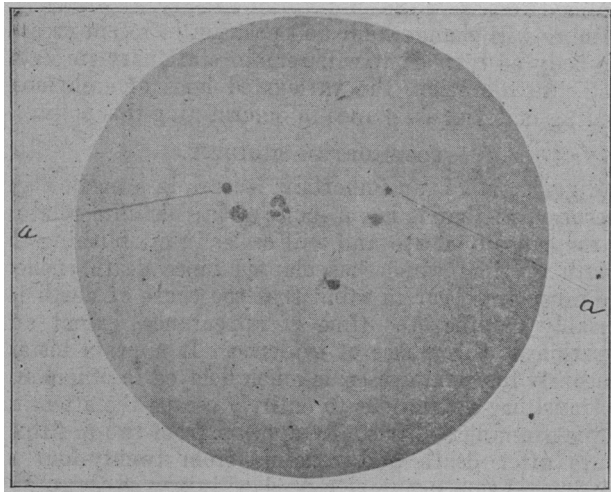


Fig. 3.—From sixth day smallpox vesicle.

boils and from several cases of cutaneous gangrene, all complicating smallpox, was examined and was seen to contain large numbers of streptococci, many in long chains.

In all of the preparations were found the so-called sporozoa described by Guarnieri and others. They were round or oval and varied in size from a large micrococcus to half the diameter of a red blood corpuscle. The average was perhaps four times the size of a staphylococcus. These bodies were found in leucocytes, but were for the most part free. Thionin stained them intensely, causing their contours to stand out in sharp relief. On coloring the bodies with Loeffler's methylene blue many of the larger ones were seen to be surrounded by a granular cell protoplasm. With both stains the coloration of these bodies was more intense than the nuclei of the leucocytes. It was noted, however, that the leucocytic nuclei did not stain in an entirely uniform manner, some being deeper in color than others and closely approaching the tint of the small round bodies. With eosin-methylene-blue these bodies did not stain differently than with Loeffler's blue. The Gram stain decolorized the bodies in the same manner as it did the nuclei of the leucocytes.

The fluid from several vaccinations was also examined and the same bodies found to be present. In fact, it would have been difficult under the microscope to distinguish between vaccinal and variolous fluid.

A thin watery fluid containing yellowish granules obtained from a fourteenth-day vaccination showed a considerable number of these bodies. This fluid inoculated on nutrient agar was proven to be perfectly sterile.

CONCLUSIONS.

The above results, it appears to me, justify the conclusion that the pustulation in smallpox is not due to secondary infection with any of the ordinary pyogenic germs, but is the result, in all probability, of the action of the micro-organism which produces the disease. Streptococci and other adventitious bacteria may be present in the late pustules, but occur only exceptionally in the early lesions. It is probable that the streptococcus plays an important part in the development of impetigo, boils, abscesses, erysipelas and gangrene which so commonly complicate smallpox.

I desire to acknowledge the valuable assistance rendered to me in carrying out this work by Drs. E. L. Graf and G. B. Walp, resident physicians at the Municipal

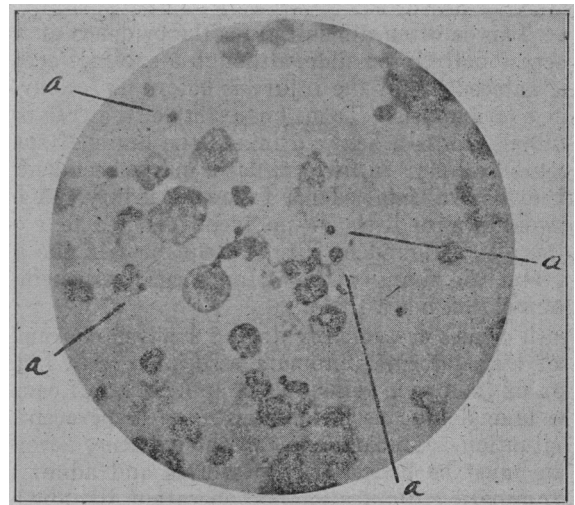


Fig. 4.—From fourteenth day vaccination.

Hospital, and Mr. J. B. Mencke, Jr., a medical student of the University of Pennsylvania.

DISCUSSION.

DR. LOUIS C. AGER, Brooklyn—I would like to ask Dr. Schamberg if he thinks the small round bodies which so many think are the small cell nuclei differ in any marked respect from what we suppose to be the white cell nuclei in other forms of pus. We often find in pus the degeneration of leucocytes, apparently with free nuclei. Is there any marked difference between these conditions?

DR. GEORGE BLUMER, Albany, N. Y.—I heard Dr. Ewing's paper on this subject in Washington, and I was under the impression that his cases were all old postmortem cases, and that would account for the large number of streptococci present.

DR. J. F. SCHAMBERG, Philadelphia—I think my information is correct in regard to Dr. Ewing's findings, as the data were taken directly from a letter received from Dr. Ewing on this subject. I am aware that in addition to the work to which I have referred he reported having found streptococci in various organs; I have also found them in the heart, spleen and other organs at autopsy.

As to the nature of the so-called "variolous bodies," and as to their identity with granules resulting from leucocytic degeneration, that is the question to be solved. You will see from the photographs that some of these granules are ex-

tremely small, not exceeding in size the large micrococci commonly found on the skin.

DR. F. F. WESBROOK, Minneapolis—Are these bodies the same as those found in epithelioma?

DR. SCHAMBERG—Probably not.

POSTMORTEM EXAMINATIONS.*

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CINCINNATI.

RESPONSIBILITIES OF MEDICOLEGAL EXAMINATIONS.

The legal aspect of postmortem examinations and reports of cases involving inheritance, crime and insurance entails responsibility second to none allotted to man in his relation to his fellow beings. The evidence elicited by medicolegal examination may very properly be regarded as the basis on which conviction or acquittal will rest in criminal cases, and the liberty or life of the defendant at bar, in many instances, is wholly in the hands of the pathologist.

Admitting the above propositions as true, the necessity of care, painstaking and thoroughness in the prosecution of this work is equally patent to physician and jurist. We are frequently asked, not only to ascertain the cause of death, but to actually fix responsibility for same. This is often difficult from the evidence at hand and occasionally impossible, although a succinct account of the antecedents of the injury is before us for investigation and analysis. Take, for example, the case of an individual who is a heavy drinker; the brain of such a subject is "watery" in life and has a marked tendency to rapid, extensive, fatal edema following injury. A blow that would cause little or no inconvenience to a well man may be followed by dire consequences in the alcoholic, and the fixing of moral and legal responsibility will annoy you not a little.

Death ensues occasionally during a physical examination of large uterine fibromata associated with inflammation of the pelvic veins, in the application of caustics to the faucial mucous membranes or after paracentesis. The situation is embarrassing in the extreme, nevertheless we must be honest with ourselves and admit that the examination, application and operation, like the blow which felled the alcoholic, were the exciting causes of death.

Persons dying suddenly with little or no history of deceased for a few days preceding death, are the most troublesome cases which confront the examiner. They are numerous in every large city, and the pathologic analysis will often tax to the utmost the acumen of the medical jurist. Unfortunately, the press and public assume in nine of every ten of the cases that some crime has been committed and the "sleuths" of the press, minions of the law and gossip of the vicinity where the corpse was found suffice to set the machinery of the coroner's office in motion. Thus we begin a legal inquiry into the cause of death, with a view to establishing the circumstances leading up to and fixing, if possible, the responsibility for same. The position in which the body lay when first discovered will assist us materially in determining the manner if not the actual cause of death.

POSTMORTEM STAINING.

In case the body has been moved postmortem staining, when present, if carefully studied, will elicit definite information. The position which the corpse occupied

likewise throws a ray of light on cause of death. The under surface of the body becomes stained first, except where pressure had existed—call them "pressure points"—and these patches remain white in the Caucasian race, and sometimes exhibit images of objects on which the corpse had rested. Much importance is given to the presence and extent of livor mortis; it is one of the signs of death and is therefore of great value in differentiating cases of suspected death from suspended animation. Too much reliance, however, must not be placed on this sign, as there are trustworthy exceptions; the writer has seen this discoloration take place several hours prior to death in a case of gangrenous appendicitis with perforation.

Postmortem staining is prone to ensue soon after death in persons afflicted with arteriosclerosis; on the other hand, it is much delayed in subjects dead of long-continued, exhaustive disease or hemorrhage. It must be differentiated from ecchymosis following trauma; this may be done in the following manner: By incising the skin covering the parts and noting the condition of adjacent tissues. In ecchymosis we find diapedesis of red blood corpuscles, while in postmortem staining we find an exudation of serum and deeply congested condition of blood vessels in the immediate vicinity. Postmortem staining will change color and position in recent cases if the body be turned; it will remain stationary in ecchymotic discoloration, the variegated hues of ecchymosis lending material assistance in determining this point.

POSTMORTEM RIGIDITY.

Rigor mortis is an important feature in a medicolegal necropsy, in that it has a more or less definite relation to the cause of death and will assist in establishing the length of time which has elapsed since death ensued. We must ever bear in mind that the cause of death invariably modifies the time of appearance, extent and duration of this phase of evidence. It appears instantaneously in certain cases, is much delayed in others and so transitory at times as to entirely escape the attention of the examiner. It usually appears from two to fifteen hours after death and remains from twenty-four to thirty-six hours. Subjects dead of injury to the spinal cord, poisoning from large doses of arsenic or carbolic acid and those dying suddenly while in good physical condition become rigid soon after death; conversely, those dead of exhaustion, hemorrhage or shock will manifest no rigidity immediately after death; it will appear late, be slight and transitory.

Our physiologists are fond of referring in their reports to the condition of the heart at time of death. They say that the animal died in systole or diastole, and attach much importance to this incident; to the pathologist it signifies the presence or absence of postmortem rigidity in the heart muscle. Thus we rationally account for those peculiar phenomena of the cadaver which ever and anon excite the wonder and astonishment of the laity, persons found standing upright, resting against some object, riding on horseback and in the attitude of prayer though cold and rigid in death for many hours. While much has been written on this early and ever-constant postmortem change, extensive research is being made and valuable evidence may be had by a study of post-mortem rigidity in any given case, it is singularly unfortunate that the one important forensic question, "At what time did death take place?" can not be answered by deductions drawn from this source. If asked by the court to testify as to the length of time which had

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