as the internal variety. In prolapsus ani the injection is made into the mucosa and deeper structures; sections of these tunics are then ligated and cut off.

In cutaneous hemorrhoids the skin is first anesthetized, then the central portion of the hemorrhoid; the tumor is then removed with a sharp knife. I say knife, because I have observed that scissors are more apt to cause pain than the scalpel. In all cases requiring deep or extensive operations the skin should be carefully and thoroughly anesthetized first, and then the deeper structures. Very fine needles are used for the skin, coarser ones for the subcutaneous tissues, and for the deeper structures still larger ones, which are blunt and have holes in the side instead of in the end, to avoid injury to vessels. It is important that the solution be warmed to the body temperature. Rapid injections should be avoided, as sudden distension of the tissues may be painful. For the same reason neither solution should be used when cold or too hot. Strange as it may seem, most patients complain of but little pain on the days after the operation. In fact, they have much less pain than after taking chloroform or ether, to say nothing of the suffering experienced by some in coming out from under the influence of the anesthetic, and the healing process seems to be much more rapid and satisfactory. This is probably because the resisting power of the patient has not been lowered with a general anesthetic, as it has been shown by George Rubin of Chicago⁸ that the administration of ether and chloroform has a decidedly detrimental influence on the natural defenses against infection.

After hemorrhoidectomy a cone-shaped dressing covered with a piece of the thinnest rubber dam is applied to the anus, and over this the ordinary dressings, all being held in position by a snugly fitting T bandage. Should the patient complain of much pain, hot compresses or a hot sitz bath may be used and will usually give relief.

I have operated on about 75 patients under local analgesia. The aggregate amount of pain has been much less than in a similar class of cases operated on under general anesthesia. In some instances the pain was quite sharp for a few moments, but nothing in comparison with the pangs, anguish and writhings occasionally seen in patients after the ligature operation for hemorrhoids under general anesthesia. In one patient I had to abandon the operation. This was purely a case of "psychic pain." I do not believe that any local analgesia can control this nervous dread and fear experienced by some patients. A hypodermic of morphin, administered 20 to 30 minutes before operating, seems partially to control the pain in some cases. Many of these patients did not have any pain worth mentioning.

CONCLUSION.

Since practice and experience have demonstrated that effective and radical treatment in this class of diseases can be accomplished in the office or in the patient's home, and without his foregoing all business and social obligations, going to the hospital, taking an anesthetic, which always occasions more or less anxiety on the part of both the surgeon and patient, and remaining there for two or three weeks, I believe that another step in advance has been made in the treatment of selected cases in the practice of proctology.

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8. Trans. Chicago Path. Soc., March 14, 1904, vol. vi, No. 4.

THE APPLICATION OF LABORATORY METHODS TO THE DIAGNOSIS OF VARIOLA.

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The rapid increase of our knowledge of infectious diseases since the middle of the last century, together with the recent advances in laboratory methods, particularly in bacteriologic and histologic technic, has had a profound influence on the accuracy of medical and surgical diagnosis.

Tuberculosis, cholera, typhoid fever, anthrax, actinomycosis, diphtheria, bubonic plague, pneumonia, bacillary dysentery, and various forms of primary and secondary wound and other infections, among the bacterial diseases; trichinosis, pébrine, malaria, amebic dysentery, filariasis, trypanosomiasis, and various forms of helminthiasis, among the diseases caused by animal parasites, may be enumerated as examples of infectious diseases for the diagnosis of which laboratory methods are in constant use. In short it may be said that, as soon as the parasitic origin of a disease is established, the demonstration of the presence of the causal agent is used as the basis of exact diagnosis. Laboratory methods are also availed of in the diagnosis of certain affections whose etiology is still undetermined, as rabies, tumors and certain diseases of the blood-forming organs for instance. The methods in use for these purposes are (1) microscopic examinations (a) of coverslip preparations, (b) of sections of tissue; (2) cultures; (3) serum reactions; and (4) animal inoculations.

Recent advances in our knowledge of variola make it possible, it seems to me, to utilize two of these methods for the diagnosis of this disease.

Councilman, Magrath, Brinckerhoff,¹ and Tyzzer,² in 1903 and 1904, published a series of articles concerning the etiology of vaccinia and variola, in the skin lesions, of which they found certain bodies situated in the cytoplasm and nuclei of the epithelial cells, and which they describe as stages in the life cycle of a protozoan parasite, *Cytorrhycles variolæ*. These bodies, of various sizes, shapes and staining reactions, occur only in the cytoplasm in vaccinia and in both the cytoplasm and nuclei in variola.

Calkins,³ using their variola material, has made out a very complex life cycle for this parasite, consisting of one cytoplasmic and three internuclear stages. He classes the organism among the microsporidia, a form of protozoa. More recently Howard and Perkins,⁴ working on material from seventeen cases from the recent Cleveland variola epidemic, found the bodies as described by Councilman and his co-workers, and confirmed their work, with the exception of one of the intranuclear stages of Calkins, which they could not find. Howard and Perkins⁴ added a second cytoplasmic stage, and according to them the life cycle of the organ-

1. Councilman, Magrath and Brinckerhoff: A Preliminary Communication on the Etiology of Variola, Jour. of Med. Research, 1903, vol. ix, p. 372. The Pathologic Anatomy and Histology of Variola, Ibid., 1904, vol. xi, p. 12. The Occurrence of *Cytorrhycles Variolæ* (Guarnieri) in the Skin of the Monkey Inoculated with Variola Virus, Ibid., 1904, vol. xi, p. 173.

2. E. E. Tyzzer: The Etiology and Pathology of Vaccinia, Jour. of Med. Research, 1904, vol. xi, p. 180.

3. Gary N. Calkins: The Life Cycle of *Cytorrhycles Variolæ*, Guarnieri, Jour. of Med. Research, 1904, vol. xi, p. 136.

4. Howard and Perkins: A Study of the Etiology of Variola, Jour. of Med. Research, 1904, vol. xii, 359.

ism, as far as is known, consists of four stages, a primary and a secondary cytoplasmic and a primary and a secondary intranuclear. They also found the bodies in the skin lesions excised during life, as well as those obtained at autopsy. So far as is known, these bodies in the skin lesions of vaccinia and variola are peculiar to, and characteristic of, those diseases. Councilman, Magrath, Brinckerhoff⁵ and Tyzzer² have found only the primary cytoplasmic stage in vaccinia.

With the foregoing facts in our possession, it is a natural step to utilize this knowledge for the diagnosis of vaccinia and variola from each other and from other diseases.

DIFFICULTIES IN THE DIAGNOSIS OF VARIOLA.

While the clinical picture of variola in its usual type, especially in well established epidemics, is clear cut and easy of recognition, it is well known that isolated cases, particularly early in epidemics, often present clinical pictures of indefinite characters and are difficult of exact diagnosis. Among the affections most commonly confused with variola are varicella, syphilis, pemphigus, impetigo contagiosa, measles, lichen, the bites of certain vermin, and certain drug rashes. Hemorrhagic variola may be mistaken for various other forms of purpura. Bancroft suggests that certain cases of variola without characteristic eruption are thought to be influenza.

To properly diagnose and isolate primary cases is of the greatest importance in the control of variola. In many instances the failure to do this is due to the ignorance on the part of a large proportion of practitioners concerning the usual and unusual clinical pictures of this disease. Largely on this account, the first cases of variola in a community go unrecognized, until after the exposure of a number of susceptible persons, and the consequent outbreak of numerous secondary foci of the disease. These occurrences often lead to disputes between physicians and health authorities. This unfortunate condition of affairs can be avoided by a ready and sure method of laboratory diagnosis.

METHODS OF LABORATORY DIAGNOSIS OF VARIOLA.

Animal Inoculation.—This method has been used heretofore to some extent. The bovinæ and rabbits will develop vaccinia after inoculation with the contents of variola vesicles and pustules. Magrath and Brinckerhoff⁵ have similarly produced variola inoculata in the monkey by inoculation with variola virus obtained from three epidemics. Park⁶ has proposed this method for differentiating variola from varicella.

Animal inoculation then, by the development of vaccinia in the bovinæ or in rabbits, or of variola inoculata in monkeys, may enable one trained in the appearances of the vaccinia and variola eruptions to make a positive diagnosis within from five to eight days, or within from eight to eleven days if one has to resort to the histologic study of the experimental lesions.

While of distinct value in certain instances, this method is too slow to be of general use. This is no doubt the chief reason why it has not been more widely adopted.

Study of Sections of Excised Skin.—With the possible exception of varicella, it is definitely known that none of the skin lesions which may be mistaken for

variola and vaccinia, show the peculiar bodies which have lately been described in these diseases and grouped into the life cycle of a protozoan parasite by Councilman¹ and his pupils,² by Calkins³ and by Howard and Perkins.⁴ In the one case of varicella from which I have had the opportunity of studying sections of the excised skin, no such bodies were found.

Whether one agrees or not that the bodies described in vaccinia and in variola by the above-mentioned observers are protozoan parasites, is not of importance for our present purpose. For this it is sufficient that these bodies, in the sequences given below, are constantly present in the skin lesions of vaccinia and variola, and are absent in other affections for which these diseases may be mistaken. As they are peculiar to and characteristic of vaccinia and variola, and as the sequence of these bodies are different in these two diseases, I, therefore, propose the microscopic examination of skin lesions excised from suspected cases of variola for the purpose of diagnosing this disease in doubtful cases. I believe that in this way a positive diagnosis can be readily made in almost all, if not in all cases, provided the technic advised below is carried out with exactness.

I⁴ have studied skin lesions excised during life from eight cases of variola in different stages of the disease, from the early papular to the pustular stage, and have found *Cytorrhycles variolæ*, in some stage of its life cycle, present in all. The cytoplasmic and intranuclear bodies are the same in all respects, whether the skin be excised during life or obtained at autopsy.

TECHNIC.

Small pieces of characteristic areas of the skin with the suspicious eruption are excised, under local anesthesia by ethyl chlorid, with the aid of a sharp knife or curved scissors. Care must be taken to obtain specimens large enough to include the whole lesion and the underlying corium. The specimens should be fixed at once in Zenker's fluid, the outside of the bottles properly disinfected, and the bottle and contents transported to the laboratory as soon as possible. Zenker's fluid is the only fixative that gives satisfactory results. It has the following composition:

Bichromate of potassium	2.5 grams
Sulphate of sodium	1. gram
Corrosive sublimate	5. grams
Glacial acetic acid	5. c.c.
Water	ad, 100. c.c.

Dissolve the corrosive sublimate and the bichromate of potassium in water, with the aid of heat; add the acetic acid just before using.

From now on the time element is of importance and the fixing, imbedding and preparation of sections must be carried out with the utmost celerity consistent with good results. If the material must be sent to a distant laboratory, the time consumed by the journey will suffice for proper fixation. For fixation in Zenker's fluid, the time for small pieces of tissue may be cut down to from eight to twelve hours. By allowing a similar time for washing, the tissue may be run through the various steps for paraffin imbedding and good sections obtained in from sixty to ninety hours from the time the skin is excised. Thin sections are necessary, and I prefer them from three to four microns thick; but good results may be had with sections of seven or eight microns in thickness. Paraffin imbedding is the only method to be relied on for this purpose.

There is little chance that frozen sections may be of

5. Magrath and Brinckerhoff: *Experimental Variola in the Monkey*, Jour. of Med. Research, 1904, vol. xi, p. 230.

6. Park, W. H.: *A Practical Method of Differentiating Between Variola and Varicella by Means of the Inoculation of Monkeys*, Trans. Assn. Am. Phys., 1902, vol. xvii, p. 208.

service, but this method might be tried. It is possible that in a certain percentage of cases curretings from the deepest part of the skin lesions spread on cover glasses or slides, fixed in Zenker's fluid and stained, may show the bodies to be described below. In the one case in which I tried it, the bodies were not found. Of course, the mercury in the sections must be removed before staining. Sections may be stained by any of the following methods: (1) Eosin and methylene blue; (2) Borrel's stain; (3) iron hematoxylin, followed by aurantia; (4) Weigert's fibrin stain, preceded by carmin. The best methylene blue and eosin method is that advised by Mallory.⁷ He stains first in 5 per cent. aqueous solution of yellowish eosin for twenty minutes, followed by Unna's alkaline methylene blue for five minutes. Differentiate and dehydrate in 95 per cent. and in absolute alcohol and clear with xylol on the slide. The methylene blue method is useful, however, only for the primary cytoplasmic bodies (see *infra*). The Borrel stain should be used as advised by Calkins.³ After hydrating the sections through progressively weaker alcohols, stain for twenty minutes in a saturated aqueous solution of magenta (Magentaroth, Grüber), wash in water and stain for five minutes in a picro-indigo-carmin solution, made by adding one part of a saturated aqueous solution of picric acid to two parts of a saturated aqueous solution of indigo-carmin. Differentiate in the alcohols, ending in absolute, and clear in xylol. In properly stained sections the red stain is completely extracted from the nuclei of the epithelial cells, the cytoplasm of which is stained a pale green.

The peculiar bodies to be described below stain brilliantly. The other staining methods mentioned may be found in any good work on laboratory technic. Councilman has obtained exquisite pictures of the different forms of variola bodies in sections stained by Weigert's method.

For a detailed description of the bodies described below the reader is referred to the articles of Councilman, Magrath, Brinckerhoff, of Tyzzer, of Calkins, and of Howard and Perkins above referred to, which are amply illustrated. I give here simply a short objective description of what I consider as necessary for the diagnosis of variola in any stage from the papule to the pustule. For this purpose I will give as nearly as is possible the relation of each sequence of bodies to the corresponding stage of the skin lesion. Any one with a fair training in pathologic or clinical microscopy, with a good light and an oil immersion lens should be able to recognize them without any difficulty, in properly fixed and stained preparations.

CORIUM. PAPULAR STAGE.

The blood vessels, especially the blood capillaries of the papillæ, are dilated, and may or may not contain an increased amount of blood, the leucocytes of which bear their normal proportion to the erythrocytes. In hemorrhagic cases, the hemorrhage from the distended capillaries is evident. In many papillary capillaries, the endothelial cells are swollen, desquamated, and increased in number. The perivascular lymphatics show similar changes and often contain large mononuclear leucocytes.

In a few cases I have found in the capillaries and between them and the membrana propria of the skin small round homogeneous deeply staining bodies identical with the early bodies to be described below in the

epithelium. No other forms of the specific bodies are found in the corium, which for our present purpose is without further interest.

EPIDERMIS. PAPULAR STAGE.

In the early papules, occasionally between but commonly within the deeper layers of epithelial cells, there are a variable number of small, homogeneous, usually round, bodies which stain deeply with nuclear dyes. These bodies in the cytoplasm, called gemmules by the advocates of their parasitic origin, are usually surrounded by small vacuoles. They may be in any part of the cytoplasm, but usually are about the nucleus. In sections stained first in magenta and then in methylene blue they stain bright red, while the nuclear chromatin stains blue. In older papules, these bodies and their surrounding vacuoles are larger—the former measuring three or more microns in diameter. They are at this stage often oval, reniform or crescentic in shape, and show differentiation with various stains.

With methylene blue and eosin, the main mass is red, with here and there unstained areas. In still older lesions the bodies are larger and show a delicate reticulum which stains, with eosin, magenta and orange. Up to this time there is little change in the epithelial cells except that those containing the bodies are swollen.

In more advanced lesions the cytoplasmic bodies are larger—from 4 to 10 microns in diameter—and very irregular in shape. In Borrel preparations they are marked by larger and smaller red dots and bars and irregular areas on a green background. About this time, fluid accumulates in the lesion and the cells are much swollen. The next forms to be found vary from six to sixteen microns in diameter, and are round, oval or oblong, and have the appearance of amebæ. With Borrel's stain (the best stain from now on), the main mass of the bodies stains green and usually numerous small, round, equidistant, homogeneous bright red dots are present. Some of the bodies at this time show a delicate red-staining alveolar meshwork. Somewhat later there are found large amebæ-like forms, in the spaces of the reticulum of which one can see numerous small, round, homogeneous deeply staining dots of the size and appearance of the earliest bodies described in the epithelial cells. These dots (called gemmules) stain with methylene blue, iron hematoxylin, magenta or fuchsin.

Similar dots may also be seen in the intercellular spaces. This entire sequence of forms is common to both vaccinia and variola. In many cases they are also found at this time in the cytoplasm of cells at the margin of the original lesion. This is believed by the advocates of their protozoan nature to be local autoinfection.

The lesion at this time contains a variable amount of fluid, which varies with the severity of the case. Many epithelial cells are swollen and some are necrotic. The vesicular stage is now well established.

VESICULAR STAGE.

When this is fully established, in many lesions few cytoplasmic forms may be found. In some lesions, however, a second cytoplasmic sequence of bodies is discernible. Of these the earliest forms so far described are small round or oval bodies staining green with Borrel's stain and yellow with the iron hematoxylin and aurantia stain. They lie in the cytoplasm, from which they are separated by a distinct membrane. Later, similar forms containing from two to four dots stain-

7. F. B. Mallory: Jour. of Med. Research, 1903-4, vol. x, p. 487.

ing with nuclear dyes are seen. The next stage in this secondary cytoplasmic sequence is the appearance of larger bodies of the same type, surrounded by a membrane, but divided into four segments by faint lines of cleavage. Neighboring cells may contain from four to eight small oval somewhat granular bodies of about the size of an erythrocyte, each containing a central chromatin-like dot. Similar forms may be seen in the intercellular spaces and in the cytoplasm and nuclei of other cells.

This completes the secondary cytoplasmic sequence, which is believed by Howard and Perkins to be the second stage in the cycle of *Cytorrhycles variolæ*. So far as is known, it is only found in well-established vesicles in variola, but never in vaccinia. The lesion at this time does not usually contain enough leucocytes to warrant calling it pustular, but this varies with the severity of the case. I will, therefore, with this explanation, describe the next sequence, the primary intranuclear stage, under the vesicle.

PRIMARY INTRANUCLEAR STAGE.

This series of forms in the nucleus is readily recognizable, and begins with the appearance, in the nuclei of the epithelial cells of the deeper rows of the variola lesion, of small bodies from three to eight microns in diameter, with granular cytoplasm and a single central chromatin-like dot. This body is identical in appearance with the end body of the last-described sequence. The smaller forms do not fill the nucleus. With the progression of the series, the intranuclear bodies are increasingly larger and more complicated in structure. The first difference in the latter respect is shown by the presence of numerous small deeply staining chromatin-like dots throughout these bodies; the next difference is marked by the occurrence of a variable number of hollow rings in addition to the dots. Later, in some forms, small peripheral vesicles, each containing a central deeply staining dot, is found about these hollow rings. In other forms the whole solid body appears to be changed into a collection of minute rings with central dots; this body has a peculiar morula appearance.

The small dots within the vesicles, on close examination with high powers, are found to be hollow, circular structures or rings somewhat thickened at one side. This third sequence is interpreted as follows by the adherents of the protozoan interpretation of these structures:

The solid mass with the central dots, which somewhat later develop numerous scattered chromatin-like dots, is the pansporoblast; the rings developing within these are the young primary sporoblasts, and the dots formed within the vesicles about these rings are the spores.

At this stage the lesion almost always contains enough cell detritus and leucocytes to be styled pustular; therefore, the fourth sequence of bodies will be described under the pustular stage of the pock.

PUSTULAR STAGE.

At this period in the evolution of the skin lesion, the superficial layers of the epidermis are markedly projected outward, with a cavity between them and the lower epidermis. This space is filled with fluid, cell detritus, leucocytes and erythrocytes in variable numbers. The cells of the deepest layers, as before, contain the characteristic bodies. The earliest form of the secondary intranuclear sequence of bodies is marked

by the presence in the nuclei of many of these cells of from one to six of the minute ring bodies met with at the end of the primary nuclear sequence. In many lesions these two sequences of forms overlap, some nuclei containing the end forms of the primary, and others the first forms of the secondary intranuclear sequence. The progress of the latter sequence is shown first by the presence of larger intranuclear ring bodies, with increased thickness of one side of the ring. After this, intranuclear rings are seen which have a single or multiple row of small peripheral vesicles. In larger forms which may nearly fill the nucleus, there is in each of these peripheral chambers a small deeply staining body identical with the "spores" of the primary intranuclear body. The bodies containing these dots are called the secondary sporoblasts, and the dots the secondary spores, which are believed to be the end forms of the life cycle of *Cytorrhycles variolæ*.

The bodies of this secondary intranuclear sequence are readily found and stain red with Borrel's stain and with fuschin; black with iron hematoxylin, and blue with Weigert's stain. With the completion of this series of forms the pustular stage is complete, and the progress of the pock is at an end. In some lesions, however, at this stage, at the border of the pock, the cytoplasm of some epithelial cells may contain the bodies described in the papule as the primary cytoplasmic sequence; this is regarded as local autoinfection. None of the bodies are found in the healing pock.

In studying the variolous lesion with the purpose of finding the above-described bodies as a basis of diagnosis, it is, of course, necessary to remember that these structures occur in definite groups or sequences, and that it is useless to look for the cytoplasmic forms (with the exception of the "local autoinfections") in late lesions or for the intranuclear forms in early lesions.

As has been stated above, it is not my purpose to give in the present paper a full description and interpretation of these bodies, and the reasons for believing them to be protozoan parasites. This has been done in another article.* I wish at this time simply to call attention to them as definite structures peculiar to variola and vaccinia on which the diagnosis of these diseases can be based, believing, as I do, that an early diagnosis can thus be made in doubtful cases, with the result of cutting short variola epidemics.

TREATMENT OF INTESTINAL AMEBIASIS IN THE TROPICS.

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The happiest results which may follow any research are those which lend themselves to practical application in the prevention or cure of disease, and nearly all of the great advances made in therapeutics are of this class.

Because the progress made in the treatment of intestinal amebiasis since its identification as an independent disease by Osler, Councilman and Lafleur has been of an empirical nature, there has been a long delay in introducing the methods most generally employed at the present time, and these are still far from satisfactory. The differences which exist regarding the treatment of the disease in various places within