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THE HISTOLOGY OF THE LESIONS OF
JOHNE'S DISEASE.

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IN view of the close relationship between tubercle bacilli and the bacilli of Johne's disease which is suggested by their resemblances in respect of morphological, cultural, and staining characteristics, the difference in the appearance and distribution of the lesions which they produce must be considered very remarkable.

In tuberculosis in cattle there appears to be scarcely any limit to what may be called the invading powers of the bacilli. If time is allowed the disease may involve almost all the organs of the chest, abdomen, and pelvis, together with their related lymphatic glands, and no tissue of the body can be said to be entirely exempt from attack.

In Johne's disease, on the other hand, the bacillary invasion appears invariably to begin and end with the intestines and the groups of lymphatic glands standing in immediate connection with them.

The contrast in the macroscopic character of the lesions in the two diseases is no less striking. In tuberculosis the lesions exhibit a power of progressive growth which enables them in many cases to attain to a very large size, and almost without exception they soon undergo necrotic and degenerative changes which entail striking alterations in their naked-eye appearance.

In Johne's disease the new tissue which is formed in the parts invaded by the bacilli is very moderate or absolutely meagre in amount, and, at least in the bovine species, the lesions never show to the naked eye any evidence of caseous or calcareous degeneration:

Finally, the nodular character of the lesions which is so common in tuberculosis is never seen in Johne's disease. In the intestine the only alteration which the eye can detect is a more or less diffuse moderate thickening of the mucous membrane, associated in the small bowel with a coarse wrinkling or ridging of its free surface. The closest inspection does not enable one to distinguish between the pre-existing normal part of the membrane and the super-added or replacing new tissue which is the cause of the thickening.

In the lymphatic glands the alterations are even less conspicuous. In those attached to the large intestine they are usually quite inappreciable, and in those of the mesenteric group there is often no more than a doubtful enlargement, without anything which



FIG. 1.

Nearly vertical section through mucous membrane of small intestine, showing the new tissue which has appeared between the glands and involved their partial destruction. For the most part this new material has already reached the symplasma stage. ($\times 100$.)

enables one to pick out with the naked eye the places at which the bacilli have been at work. The only other alteration observed is an appearance suggestive of œdema, an abnormal quantity of liquid, thin and watery in appearance, escaping from the surface exposed by an incision.

The principal purpose of this article, however, is not to describe the macroscopic appearance of the lesions in Johne's disease, but to examine their histology, and to compare that with the histology of tuberculous lesions in animals of the same species—the bovine.

Lesions in the Intestines.

The appearance presented by sections through the bowel wall when examined under a low magnification presents little variety

except with regard to the extent to which the disease has involved the mucous and submucous coats. It is probable that the former is always the first to be attacked, but where the wall of the bowel has been recognisably thickened to the naked eye the submucous coat is always more or less extensively affected. One can also find indications that the disease may spread along the textures of that coat, and then invade the muscularis mucosæ and spread inwards between the tubular glands. The muscular layers of the bowel wall are usually unaffected, but minute foci of disease are sometimes present in the connective tissue between the bundles of muscle cells, and even in the serous covering of the bowel, when the submucous is extensively involved.

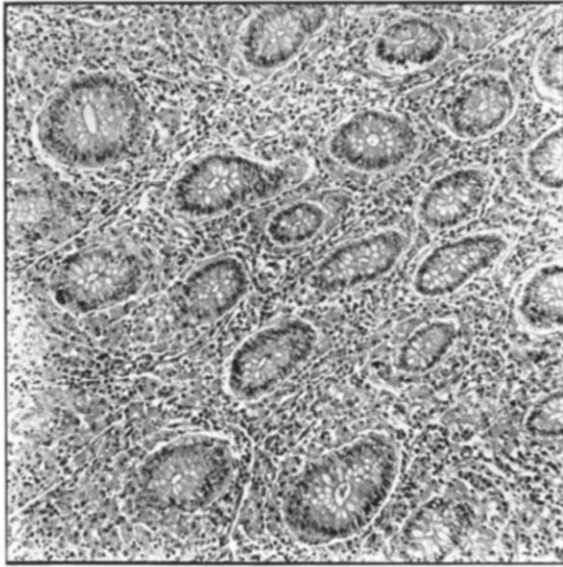


FIG. 2.

Oblique section through mucous membrane, showing different stages in the formation of the new material between the tubular glands. ($\times 100$.)

Microscopic examination also reveals the fact that many of the villi are enlarged and more or less distorted.

In all these positions the most casual inspection shows that the departures from the normal appearance of the parts is mainly due to the formation of a new tissue which has a diffuse, as opposed to a tubercle-like, distribution (*see* figs. 1 and 2). In this respect the lesions of Johne's disease in the intestine are in contrast with the generality of tuberculous growths, but closely resemble those of tuberculous mastitis in the cow. It is the structure and mode of development of this new tissue which have now to be considered and compared with what occurs in tuberculous growths.

The First Stage, or Stage of Cellular Infiltration.—This is in one sense the most important stage, as it is the one about which there is most room for difference of opinion.

It is characterised by the appearance, at the place where the disease is beginning or extending, of increasing numbers of what may be termed foreign cells—that is to say, of cells not normally present in the part. The recognition of the foreign characters of these cells, and also the determination of their origin, is easiest in such a position as the one shown in fig. 5. The disease has there made its appearance in the submucous coat, at a place where its normal tissue was composed of a sparsely nucleated, delicate, areolar connective tissue. In fig. 6 it is seen that this tissue has become more or less crowded with comparatively large uninuclear cells, between which the fine stroma of the part is still distinctly recognisable.

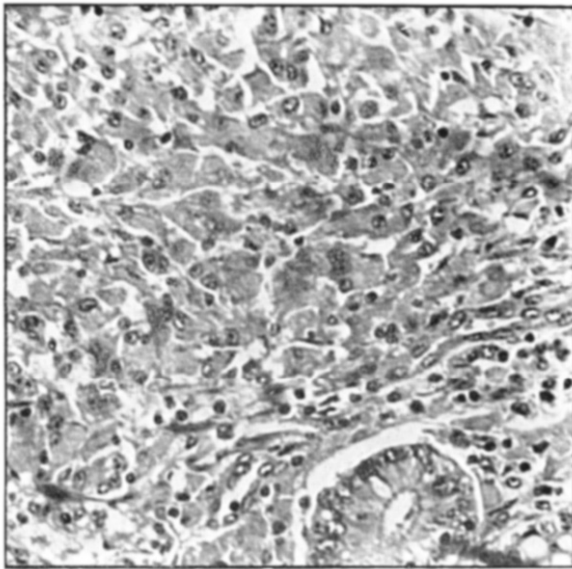


FIG. 3.

Shows cellular material which has been infiltrated between the glands, of which one is seen at the bottom of the fig. For the most part the cells have passed into the symplasma condition. ($\times 310$.)

That these new cells correspond with the epithelioid cells or plasma cells of tuberculous new tissue is indicated by their shape, structure, and staining reactions.

It may therefore be stated briefly that, with regard to their initial structure, the intestinal lesions in Johne's disease have a histology absolutely identical with that of tuberculous lesions in general.

As I have recently dealt at considerable length with the nature and origin of plasma cells as they occur in tuberculous new tissue (*see this Journal*, Vol. XXX., p. 147), it is not necessary to discuss these points at any length here. It may suffice to say that a careful study of the plasma cells occurring in the intestinal lesions in Johne's disease gives the strongest support to the view that they are derived from the normal fixed connective tissue cells, and not from lymphoid

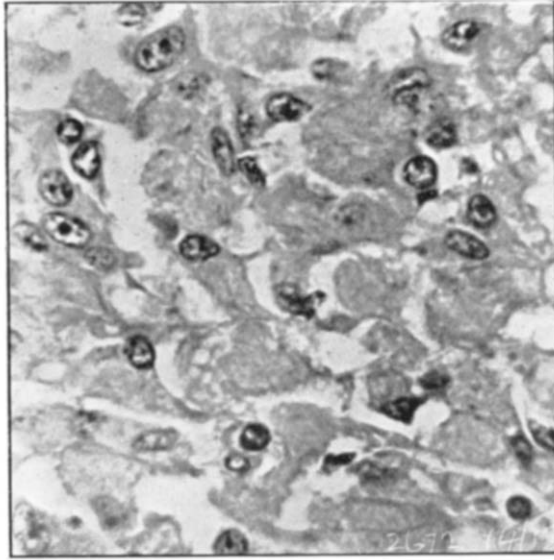


FIG. 4.

From the centre of a greatly enlarged villus. The tissue has passed into the syncytium stage. ($\times 560$.)

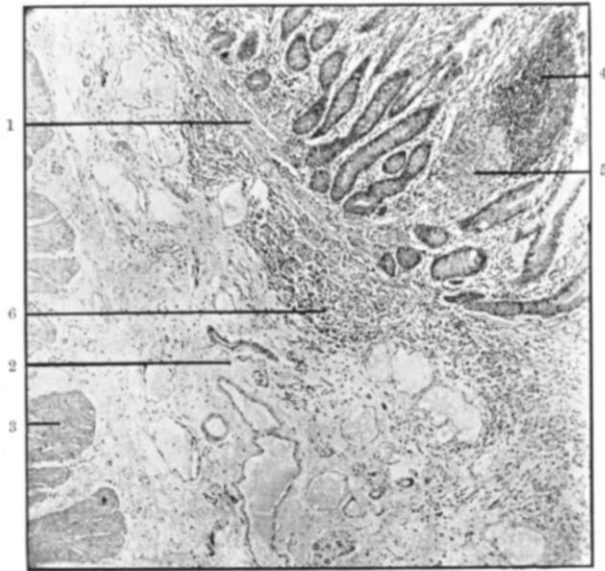


FIG. 5.

Nearly vertical section through the bowel wall. ($\times 84$.)

1. Muscularis mucosæ, which towards the right has become largely destroyed.
2. The normal delicate tissue of the submucous coat, showing sections of vessels.
3. A bundle belonging to the inner muscular coat.
4. A collection of lymphoid cells constituting a solitary gland in the mucous membrane.
5. A diseased area which has passed into the syncytium stage.
6. Margin of a diseased area infiltrated with plasma cells (*see next fig.*).

or wandering cells of any kind. In the submucous coat one can trace all transitional stages between perfectly normal cells attached to the collagenous fibres of the connective tissue of the part and the most typical plasma cells in their neighbourhood.

Furthermore, it may be added that in the intestinal lesions of Johne's disease, as in tuberculosis mastitis, leucocytes of the polynuclear type are generally entirely unrepresented, and that the presence of any of the other varieties of leucocytes must be regarded as quite accidental. Mononuclear leucocytes, especially those of the lymphocyte type, occur normally in the villi, and may therefore be found in lesions in these, or in aggregations of plasma cells between the tubular glands. Considerable numbers of eosinophile leucocytes are also sometimes present in the mucous membrane; but none of these

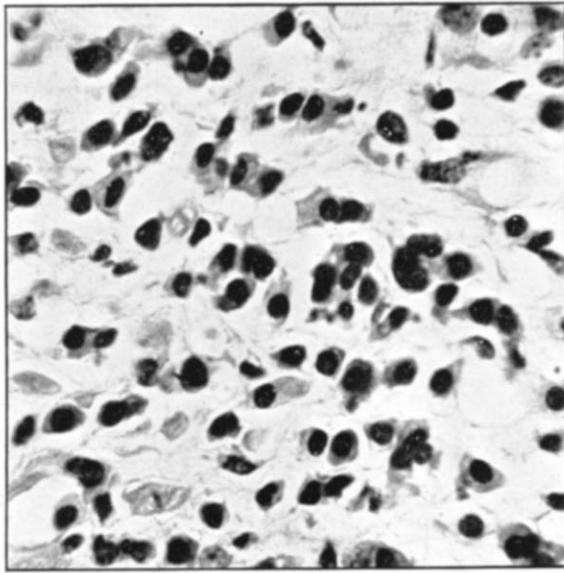


FIG. 6.

From the spot marked "6" in the preceding fig. ($\times 750$.)

Shows the infiltration of the delicate tissue of the submucous coat with plasma cells.

cells are ever specially numerous at or near the places where the bacilli have made their appearance.

It remains to notice a very important fact in connection with the cellular infiltration which occurs in the lesions of Johne's disease. It is that typical plasma cells are never found in large numbers except at the very inception of the infiltration. When the latter has advanced so far as to entail the destruction of the pre-existing normal tissue (second or histolytic stage), the great majority of the new cells have characters which distinguish them from true plasma cells as defined by Unna, Marschalko, and others. They are larger and more regularly vesicular, and their cytoplasm does not stain electively with alkaline methylene-blue or pyronin. More frequently than in the case of typical plasma cells, the nucleus is excentric,

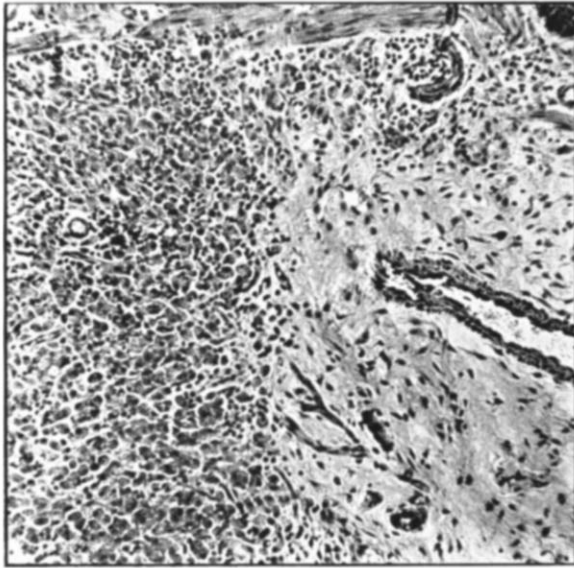


FIG. 7.

Section through the submucous coat. (\times about 80.)

At the upper part of the fig. remains of the muscularis mucosae are seen. The right half of the fig. is occupied by nearly normal connective tissue, and in the left half this tissue has been destroyed and replaced by plasma or epithelioid cells. In between these two parts plasma cells have made their appearance in the connective tissue.

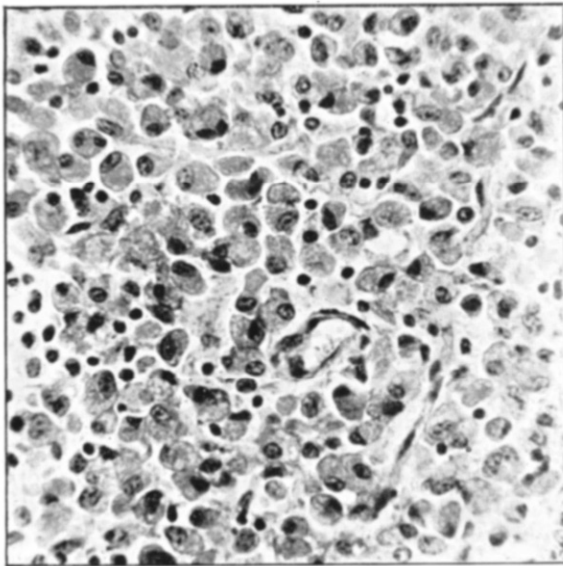


FIG. 8.

From the submucous coat. (\times 400.)

The fig. shows the epithelioid cells (altered plasma cells) which have replaced the normal tissue of the part. Sections of intact vessels are seen in it. A parallel section in the same series when stained with carbol-fuchsin showed that the epithelioid cells were all more or less filled with bacilli.

and in fact near the surface of the cytoplasm. Exactly similar cells are to be found in tuberculous lesions, but seldom in such large numbers as in the lesions of Johne's disease. The appearance presented by these cells is well seen in fig. 8. These large epithelioid cells are altered plasma cells, and the transformation is brought about by the bacilli multiplying in their cytoplasm.

The fixed connective tissue cells appear to be the only textural elements which are stimulated to proliferate, and thus to produce the plasma cells in tissues invaded by the bacilli of Johne's disease. The other cells appear to maintain an attitude of indifference, and either disappear or become incorporated with the symplasma of the third stage. Moreover, the connective tissue cells frequently appear to play an almost entirely passive part. One finds places where

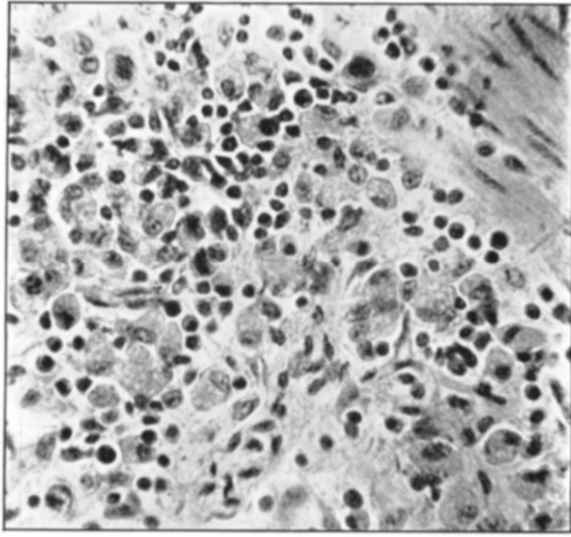


FIG. 9.

From a spot towards the edge of the infiltrated mucous membrane shown in fig. 7.
($\times 400$.)

The fig. shows transitions from plasma cells to large epithelioid cells. At the right upper corner remains of the muscularis mucosae are seen.

many of these cells have been invaded by the bacilli while still attached to the connective tissue stroma, and in such a case no proliferation follows. The cell substance becomes distended with the multiplying bacilli, and the nucleus, while still retaining its oval or elongated shape, becomes enlarged and distinctly vesicular in appearance, and its chromatin stains less deeply than before. This type of lesion is shown in fig. 10.

The Second or Histolytic Stage.—This stage is marked by the gradual disappearance of the pre-existing normal tissue elements at the place which has become crowded by plasma cells. There is nothing to distinguish this tissue destruction from that which occurs in tuberculosis except that it appears to be less intense, and it is noteworthy that even the smallest vessels are often

spared and apparently capable of carrying on the circulation in places from which the other elements have completely disappeared (see fig. 8)

It may be remarked that the destruction of the tubular glands and the structural alterations which occur within the villi must interfere both with digestion and absorption, and be held partly responsible for the progressive emaciation which is such a pronounced symptom of the disease.

The Third or Symplasma Stage.—In the article on tuberculous mastitis already cited, I suggested that the term symplasma should be used to designate a peculiar condition which occurs with great constancy in tuberculous lesions, but which had previously not been sharply distinguished from the stage of necrosis and caseation.

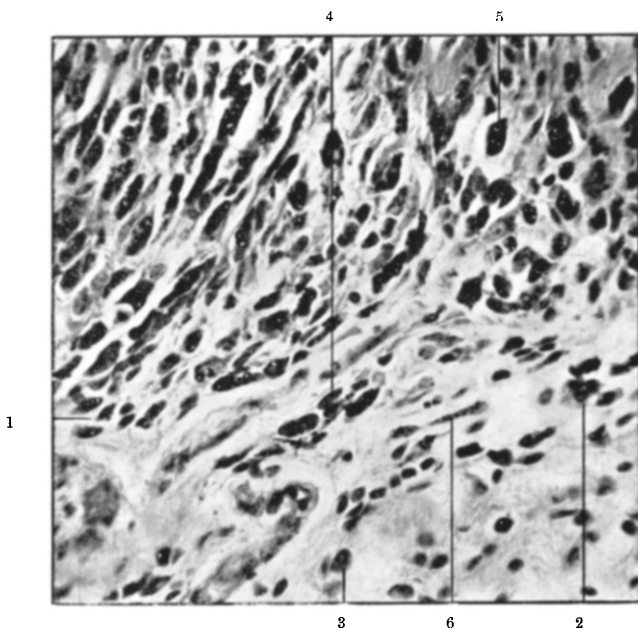


FIG. 10.

From the submucous coat ($\times 400$).

The section was stained with carbol-fuchsin, decolourised with acidulated alcohol, and counter-stained with methyl-green pyronin solution. All the dense, black, unmarked spots in the figure are masses of bacilli.

1. The two cells immediately above and below the point where the line ends are plasma cells. The object immediately to the right of the end of the line is a group of bacilli. 2. A group of plasma cells. 3. A single plasma cell. 4. A group of spindle-shaped cells filled with bacilli. 5. A mass of bacilli in a cell. 6. A spindle-shaped fixed connective tissue cell containing bacilli.

This condition results from an irregular and incomplete fusion of the bodies of the plasma or epithelioid cells, leading to the formation of a spongework or coarse reticulum without any definite structure. The nuclei of the cells which have thus become partially fused are imbedded in the substance of this reticulum or lie apparently free in its meshes. In tuberculous lesions the usual fate of the parts presenting this appearance is to undergo actual necrosis,

which in turn is followed by caseation, but there is often evidence that the symplasma stage lasts with little or no alteration for a considerable period, and that the tissue showing this partial fusion of the cells and obliteration of their outlines retains its vitality. It is the last-mentioned fact which makes it necessary to separate the symplasma stage of tuberculous lesions from the stage of necrosis and caseation; and, as will be shown immediately, the necessity for regarding the symplasma stage as a distinct one is still greater in the case of the lesions of Johne's disease.

In sections so thick that they include more than one stratum of cells the part of a lesion which is in the symplasma stage generally appears as a solid nucleated mass in which the outlines of the originally separate cells have been obliterated. The actual condition of the

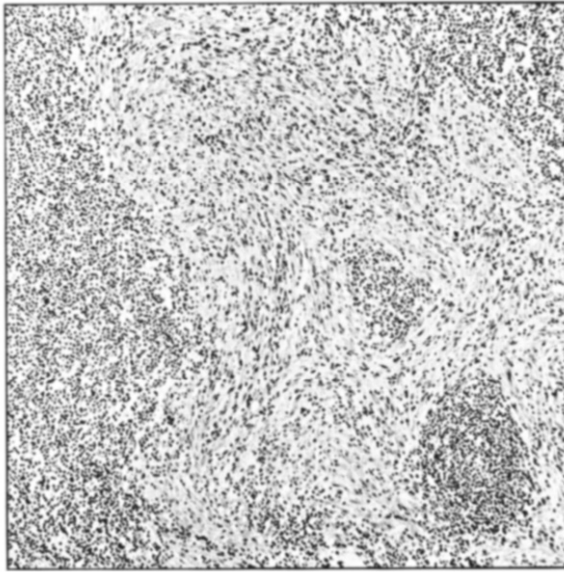


FIG. 11.

From a diseased mesenteric lymphatic gland. ($\times 84$)

The lighter tissue which occupies the great part of the field is the new tissue in the symplasma stage. Note the resemblance to spindle-celled inflammatory tissue. The rest of the field is occupied by the remains of the normal lymphoid tissue of the gland.

part is seen only in thin sections, which show that the fusion is incomplete, leaving numerous clefts and channels between the cells (*see fig. 3*).

This is the condition in which the great bulk of the new tissue is found in the lesions of Johne's disease, because, contrary to what is generally the case in tuberculous lesions, it represents the final stage. That the cells in this condition are not devitalised hardly needs to be argued.

The fact that both in the mucous and submucous coats of animals that have been visibly ill for many months, and actually infected for as long as two years, the bulk of the new tissue is always found

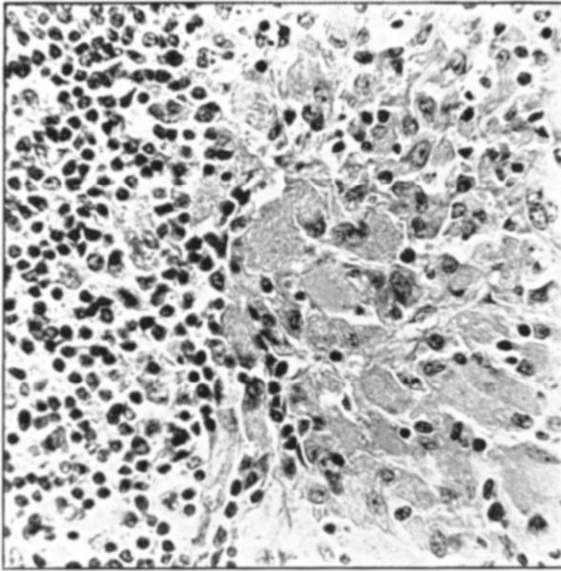


FIG. 12.

From a lymphatic gland. ($\times 400$.)

At the left of the fig. is seen remains of the normal lymphoid tissue, and the rest of the field is occupied by new tissue in the symplasma stage.

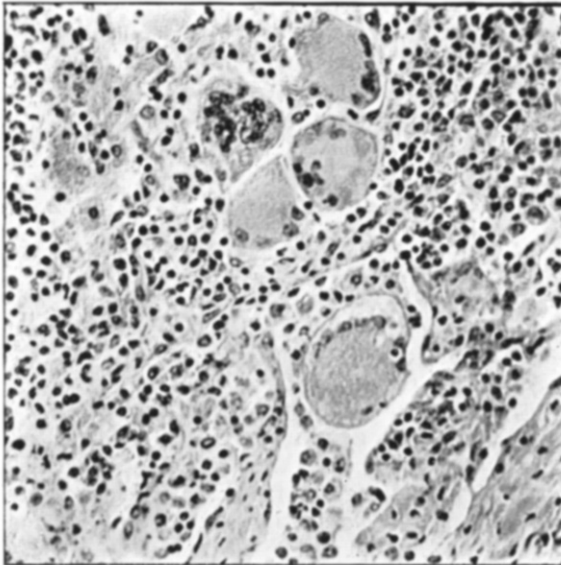


FIG. 13.

From a lymphatic gland, showing a group of giant cells. ($\times 300$.)

in this symplasma stage is quite inconsistent with the view that the parts so affected are dead.

At least in the bovine species, the symplasma stage of the lesions of Johne's disease appears never to pass into a condition of actual necrosis or caseation. This is by far the most striking difference between the lesions of Johne's disease and those of tuberculosis.

It is not to be understood that the parts which have passed into the symplasma stage are always entirely composed of altered and partially fused plasma or epithelioid cells. The contrary is the case, for it is the presence at any point of large numbers of bacilli that determines the formation of the symplasma, and, apparently in consequence of the rapid multiplication of the bacilli, the change

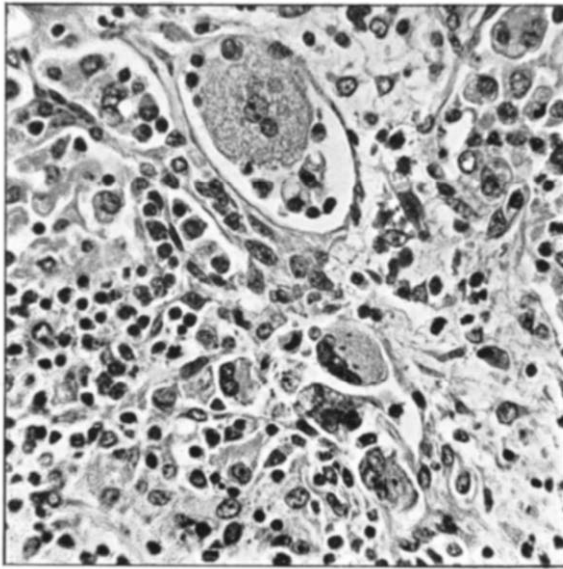


FIG. 14.
Shows formation of giant cells. ($\times 400$.)

may set in before the normal tissue of the part has disappeared, and even without any abundant antecedent accumulation of plasma cells. In such cases the surviving structures are included in the symplasma, and this contributes to the variety in the appearance of the nuclei present in it.

It is also not to be understood that no cell necrosis or destruction occurs during or after the formation of the symplasma. The masses of bacilli are often so great that they actually form a considerable proportion of the symplasma—a fact which is very striking when one compares two contiguous sections in a series, one stained only with hæmatoxylin and eosin and the other with carbol-fuchsin and methylene-blue. It then becomes apparent that the larger masses of bacilli must have entailed the destruction of at least several cells. The fact remains, however, that even in close proximity to

such large clumps of bacilli there is nothing deserving the name of necrosis.

The Fourth Stage or Stage of Giant Cell Formation.—Except for convenience of description, there is hardly any reason for regarding the formation of giant cells as constituting a distinct or separate stage in the lesions of Johne's disease. The subject will be dealt with at greater length in connection with lesions in lymphatic glands, and here it need only be said that in the intestinal lesions giant cells are always few in number compared with what is the rule in tuberculous lesions in cattle. When present they may be found in the lesions in any part of the mucous or submucous coats, including the villi.

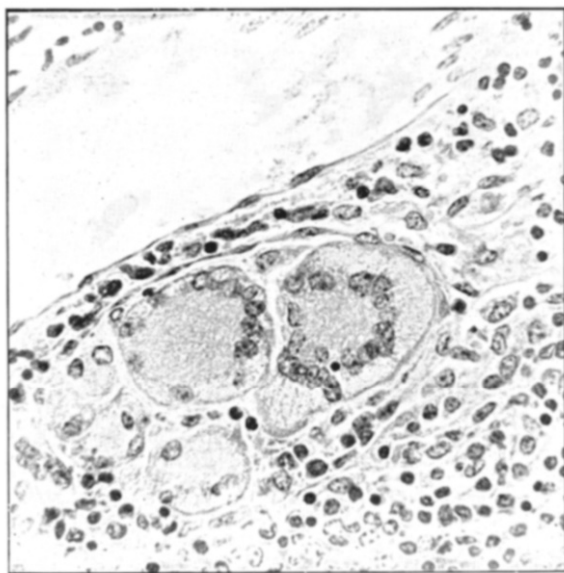


FIG. 15.

Shows a group of giant cells in what appears to be a lymphatic space or vessel. The vessel which occupies the upper part of the fig. is a vein. ($\times 400$.)

Lesions in the Lymphatic Glands.

It must be admitted that the alterations which occur in lymphatic glands are less easy to decipher than those which develop in the mucous or submucous coats of the intestine, particularly with regard to the source of the plasma cells. This difficulty arises from the densely cellular character of the normal tissue of the glands and the absence of any considerable amount of connective tissue except in the capsule and trabeculæ, which are seldom found to be invaded by the bacilli. It is in the lymph channels and the lymph nodes or follicles that the disease begins, and in these positions it is seldom or never possible to find such clear indications with regard to the origin of the new cells.

When one examines under a low magnification a section stained

with hæmatoxylin and eosin from a manifestly enlarged gland, the alterations that immediately strike the eye are: (1) the obliteration of the lymph paths under the capsule and in the cortical substance generally, and (2) the disappearance of a larger or smaller amount of the normal lymphoid tissue, and the substitution for it of a new material which is much less richly nucleated and which often has an appearance suggestive of the spindle-celled stage of inflammatory connective tissue (*see* fig. 10).

Except where they meet this new tissue, the remains of the lymph follicles appear quite normal, and some of them may show the normal lightly-stained germ centres.

Examination with a higher power shows that this new tissue in

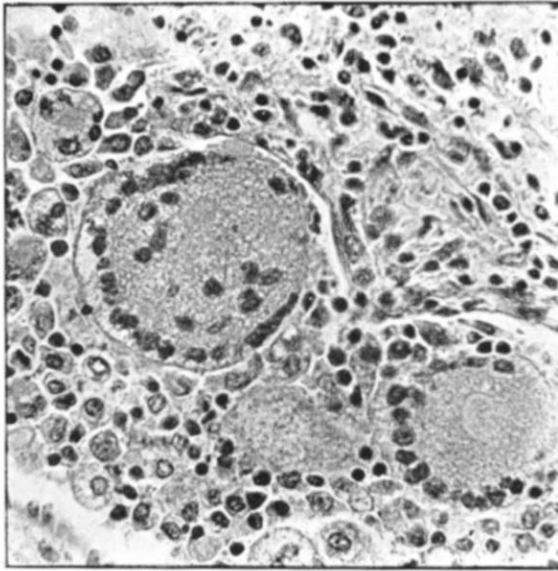


FIG. 16.

From a lymphatic gland, showing two giant cells surrounded by epithelioid and plasma cells. ($\times 400$.)

the gland is composed of cells in the symplasma stage. The identity of this condition with the symplasma stage of the lesions in the mucous membrane of the intestine will be obvious on comparing figs. 3 and 12.

In many of the bands of this new tissue the partially fused plasma or other cells have become more or less spindle-shaped, and many of the nuclei are also oval or elongated; it is this fact that gives the material a superficial resemblance to young cicatricial tissue.

Here and there in those parts of the lesions which have reached the symplasma stage one may find groups of typical plasma cells, and such cells are always to be found at or within the edge of the lymphoid tissue where it meets the new tissue.

Except in the parts adjacent to the tissue in the symplasma condition, the remains of the lymph follicles generally appear normal,

and appropriate staining shows few or no plasma cells. This applies also to the germ centres.

Appropriate staining also shows that the only parts of the gland in which the bacilli are abundant are those in the symplasma stage.

As might have been expected, the lesions appear to begin in connection with the lymph sinus beneath the capsule, and to spread gradually through the cortex. Even when the latter is extensively diseased the medullary tissue of the gland may be quite normal, but sometimes numerous plasma cells are present in it.

The peripheral lymph sinus becomes obliterated by the accumulation of cells in it. These include lymphocytes, typical plasma cells, swollen "epithelioid" cells, and even giant cells. Similar collections of cells appearing as actual microscopic "tubercles" are sometimes found in afferent lymph vessels in the substance of the capsule.

Giant cells appear to be more frequently formed in the mesenteric and other glands than in the intestinal lesions. They differ in no respect from those formed in tuberculous lesions, except that they less frequently show the so-called branches, and are more frequently surrounded by plasma and epithelioid cells which have not yet passed into the symplasma stage (*see* figs. 12 and 15). They are usually much less numerous than in tuberculous lesions in lymphatic glands, and this applies especially to the parts that have passed into the symplasma stage. Not rarely cells are found lying in spaces with a definite endothelial membrane—probably a dilated lymph sinus (*see* figs. 14 and 16).

Like the giant cells of tubercle, those found in the lesions of John's disease appear to be formed by passive fusion and degeneration of the cytoplasm of previously independent plasma and other cells.

HABRONEMIC CONJUNCTIVITIS.

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Introduction.

THE occurrence of habronema in the skin and subcutaneous tissues has appeared of recent years to be more common, or, at least, it has been much more frequently noticed, and may be assumed to be becoming more widespread, more various in its manifestations, and probably of more importance in its effects.

As mentioned in Railliet's report (1), Ercolani was the first to discover in 1859 a larval nematode (Habronema megastoma?) in the subcutaneous fibrous tissue of some of the skin lesions which in the horse are generally known as summer sores or granular dermatitis. Others, including Buffard, Rivolta, Fayet, and Moreau, have also described similar conditions. In India a somewhat similar condition is often confused with other cutaneous lesions caused by moulds, and possibly other agents, under the general term bursati. More recently the work of Descazeaux (1) on Esponja or Summer Sores of Equines in Brazil has been the subject of the report by Railliet mentioned above.