A Contribution to the Occurrence of Actinomycosis in Bovines, Associated (a) with the Peritoneal Cavity, (b) with the Testicles.

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A NUMBER of specimens found in connexion with the peritoneum and testicles of bovines were forwarded from the abattoirs in Durban. These specimens were taken from animals which were brought from Rhodesia and slaughtered for export purposes. Such cases have not yet been recorded in the Union of South Africa. On microscopical examination here it was found that these specimens were, in the majority of cases, caused by the ray fungus.

It is proposed to consider the material examined, under the

following headings: -

(a) Macroscopical and microscopical description of the lesions observed, in the peritoneum of two specimens examined,

viz., 5204 and 4977.

- (b) Macroscopical and microscopical description of the lesions seen in the testicles. In this instance a number of specimens were examined, viz., 5209, 5433, and 5435. The last two numbers included several testicles collected from a number of animals.
- (c) Discussion.

A.—Macroscopical Appearance of Specimen No. 5204.

The exact locality of the portion of the peritoneum is not clear from the specimen forwarded. It is of the nature of a thin sheet-like structure, with a good deal of elastic tissue, measuring about  $20 \times 12$  cm. It is covered by endothelium and therefore smooth, and associated with part of it, there is a good deal of fat. It shows numerous nodular swellings, some are pedunculated, others sessile. They vary in size from spherical nodules 3 mm. in diameter to a large nodule  $4\frac{1}{2}$  cm. in diameter. The majority of them, however, are about 1 cm. in diameter.

The nodules resemble to a certain extent, "grapes" of tuberculosis. They are completely circumscribed and show a well-formed glistening capsule, and the centre is made up partly of necrotic material and partly of granulation tissue (see the large nodule in plate I). The necrotic material is of a greyish white caseous nature. The connective tissue is in the form of a fine network, and interspersed between it are opaque areas which vary in colour from a pale yellow-

ish grey to a light brown.

Macroscopical Appearance.—Specimen No. 4977.

A portion of the peritoneum which shows several protruding nodules varying in diameter from 2 mm. to 1 cm. They are almost spherical and possess a glistening white capsule resembling to a large extent those seen in plate I and described above.

#### Microscopical Appearance of Specimen No. 5204.

Fibrous connective tissue capsule surrounding an irregular connective tissue stroma-like mass, in which fibrillae are somewhat irregularly arranged, and in places more are present than in others. In between the connective stroma there are several foci of the nature of fungus-like centres surrounded by cellular elements. These centres appear in groups, several of these structures lie together irregularly arranged, and of different sizes. These fungus-like structures have a peculiar serrated cantour but with rounded points and with zones stained of a different intensity. The periphery, somewhat narrow, is stained intensively blue with Giemsa, and on the outside of it a light pink-coloured fringe can be made out, and in places reveal the club-formation of true actinomycosis. The intensely stained portion, on higher magnification, show it to be made up of filaments or hyphae having a granular appearance. Some of the granules are more intensely stained and continued beyond the contour of the filaments or hyphae. The part lying central to this dark peripheral portion just described above, is composed of large granular, irregular, light pink-stained masses in which no structure can be identified. The large fungi-like masses may contain in the interior secondary smaller fungi-like structures, more or less of a similar appearance, except that there is less of the granular homogeneous pink-stained central portions present. These fungi masses are surrounded by polymorph-nuclear cells, of which the majority are neutrophiles, only a few eosinophiles were identified. There are also some round cells present. To the outside of these centres (i.e. fungus and cells) there follows a zone of round cells, and what appear to be multiplying fibroblasts. These either merge into a contiguous focus (fungus and cells), or pass into the connective tissue stroma already described.

### Microscopical Appearance of Specimen No. 4977.

This shows foci of several kinds:—

(1) Ray fungus as a wave-like mass lying in a fairly extensive broad centre of neutrophiles. These foci dominate the picture and few

round cells can actually be made out.

(2) Small ray fungi surrounded by cells which are of the nature of epithelioids some with several nuclei, almost simulating foreign body giant cells. Only a few neutrophiles present, and this central mass merges directly into the granulation tissue.

(3) Some ray fungi at one part of the periphery show appearance

of (1) and at another part (2) type of lesion.

(4) Some fungi are surrounded by epithelioid-like cells, as above, several rows deep, and this is followed by a fairly well-marked zone of neutrophiles.

# B.—Macroscopical Appearance of Specimen No. 5209.

Portion of an atrophied testicle easily recognized by the presence of the external cremaster muscle. These are pear-shaped—the base is represented by the testicle. It shows a much-thickened capsule which is composed of glistening greyish white connective tissue, in some places 1 cm. thick. This capsule encloses a centre which is about  $2\frac{1}{2}$  cm. in diameter. This shows a fine fibrous network, enclosing numerous irregular spaces (porous), in which one finds a greyish opaque crumbling material studded with pale yellowish gray granules.

#### Microscopical Appearance of No. 5209.

Exactly as described for specimen No. 5204 except that in places the fringe of clubs are very well differentiated and well stained. These clubs are fairly large, in diameter about the size of a lymphocyte, oval, and in some a well-defined stalk could be differentiated. (See plate III, fig. B.)

#### Macroscopical Appearance: Specimen No. 5433.

No. 1.—Testicle—bovine shows a portion of the cord with the tunica vaginalis which is smooth and glistening, and attached to it there are the remains of the external cremaster muscle. The testicle is much reduced in size. Its largest diameter is only 3 cm., in width 2 cm., and in depth  $\frac{1}{2}$  cm.

On section of the testicle one can recognize three zones (i) the capsule, or the outside, which varies in thickness from 1-3 mm., (ii) an intermediary zone which is greyish, semi-transparent, and (iii) a central zone which is for the most part of a dark brown colour, more or less opaque, but in which small areas of a pale, yellowish brown colour can be recognized.

#### Macroscopical Appearance: Specimen No. 5433.

Nos. 2. 3, 4, 5, 6, and 7.—Testicles are much reduced in size, show a well-defined capsule which surrounds a homogeneous-like cheesy centre, greyish yellow in colour. In some the capsula show small reddish areas, i.e. haemorrhages. No fungi detected.

#### Microscopical Appearance of Specimen No. 5433.

No. 1.—Shows a well-marked fibrous capsula corresponding to that of the testicle with well-defined blood vessels in it, and here and there accumulations or round cells. These accumulations of round cells become more evident towards the centre and practically replace all fibrillae. No trace seen of testicular tissue. Embedded in these masses of round cells are fungus-like structures immediately surrounded by a zone of neutrophiles.

The fungus-like structure is the same as described for specimens Nos. 5204 and 5209. Here and there they show well-defined clubs.

# Microscopical Appearance of Specimen No. 5433.

No. 2.—Well-defined capsule of the testicle in which there is here and there slight accumulation of round cells. In the centre can be made out the outline of the seminiferous tubules but no nuclei seen (karyolysis), and the whole stains a homogeneous pink colour.

No. 3.—The same as No. 2, except that between the capsula and the necrotic parenchyma there is extensive haemorrhage, encroaching

markedly on the remains of the parenchyma.

No. 4.—Same as No. 2, except that the round cell accumulations in the capsule are more defined, and these necrotic tubules lying in the vicinity of the capsule, show commencing calcification, and in some of the tubules the whole of the tissue is involved in this process.

No. 5.—Is involved in partial necrosis and in places extensive

indurative changes are present.

No. 6.—Same as No. 4, except that calcification is not so evident. No. 7.—Same as No. 4.

#### Macroscopical Appearance of Specimen No. 5435.

Several testicles, which show marked athrophy were examined, three of these are presented as Nos. 1, 2, and 3 (see plate II).

No. 1.—Is  $4\frac{1}{2}$  cm. and  $2\frac{1}{2}$  cm. It is more or less oval in shape and shows a capsule, on the inside of which there is a small granulation zone, which at its widest part is 2 mm. The rest of the testicle consisted of an opaque greyish brown homogeneous cheese-like material.

No. 2.—Is about 5 cm. in length and  $3\frac{1}{2}$  cm. in diameter, made up of granulation tissue in the form of a fibrous network, and interspersed in the stroma are numerous greyish opaque foci, varying in size from a pin's head to an area 2 cm. in diameter, the centre of

which is greyish-white homogeneous and opaque.

No. 3.—The remains of a testicle 5 cm. in length and 3 cm. in diameter—more or less divided into an upper 3rd which shows (see plate II) a connective tissue stroma in the form of a network, and in the meshes of which are irregular, brownish, opaque areas. The rest, i.e. the other  $\frac{2}{3}$  is made up of an opaque homogeneous pale yellowish grey substance.

#### Microscopical Appearance of Specimens No. 5435.

No. 1.—Extensive necrosis of the centre mass of the testicle, and this is surrounded by the capsule which on its medial surface shows granulation tissue.

No. 2.—Granulation tissue has replaced the necrotic central mass to a large extent; only here and there remains of necrotic foci are

identified.

No. 3.—The greater portion of the centre of the testicle is made of necrotic remains of the tubules. At the one pole there is a granulation tissue mass in which are embedded several ray-fungi centres.

#### C.—Discussion.

Necrosis, and in some instances calcification was observed in the testicles examined. These changes were accompanied by entensive diffuse atrophy of the testicle, as a result of which these organs were enormously reduced in size. These lesions were undoubtedly associated with castration done with the Burdizzo pinchers. This operation in the majority of the specimens examined brought about necrosis, and in some instances calcification besides the atrophy, but some of the testicles showed definite lesions of Actinomycosis as well. It would appear as if the ray-fungus was probably associated with the injury caused by the pinchers, and that the casual organisms in that way gained a port of entry through the skin into the tissues.

As far as could be ascertained Actinomycosis has not yet been described in connexion with the testicles of the bovines. Joest mentions that it was observed by Gorig and Kowalewsky as a rare occurrence in boars ("Spez., path., Anatomie der Haustiere," III

Band, 1 Halfte, seite 156).

The occurrence of Actinomycosis in connexion with the peritoneum of bovines does not seem to be of such a rare occurrence. Joest describes primary and secondary lesions (Joest: "Spez. Path. Anatomie," II Band. seite 436), and maintains that primary Actinomycosis in the peritoneum may arise through injuries of the abdominal wall, but cases occur in which the port of entry of the ray

fungus could not be established. Unfortunately, from the information on hand it is difficult to trace the source of infection in the two cases of multiple Actinomycosis of the peritoneum described above. These lesions were observed in apparently clinically healthy animals slaughtered for export purposes. There is a possibility that the lesions in the peritoneum may have a connexion with bruising produced by the Burdizzo pinchers, with subsequent infection. This infection then proceeded via the vaginal canal.

These lesions in the peritoneal cavity are very characteristic and are of interest, in that they resemble the "grapes" of tuberculosis to a certain extent. Joest drew attention to this in his description

of Actinomycosis in connexion with the peritoneal cavity.

As regards the causal organisms in the above cases of bovine Actinomycosis it may be stated that they belong to the streptothrix group.

Bossworth, in his paper on the causal organism of Actinomycosis (Journal of Comp. Path., Vol. XXXVI, No. 1, page 1), draws attention to the two groups of organisms that may be responsible for similar lesions.

This streptothrix is well depicted in plate V, in which the branching arrangement of the Gram-stained filaments or hyphae can be detected, also the formation of clubs, the densely intertwined mass of filaments, and the granular appearance of some of these filaments.

Unfortunately the material forwarded was fixed in formalin and therefore not available for cultural studies.

#### PLATE I.

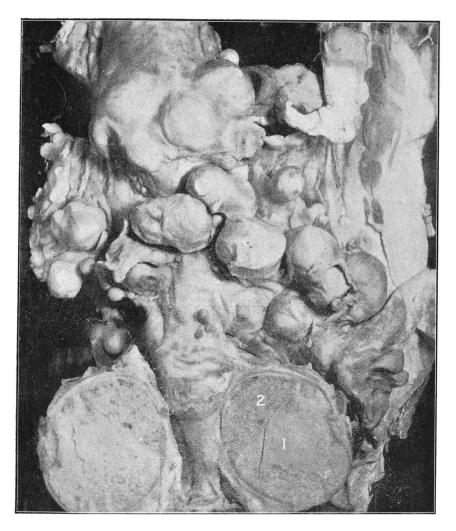


Fig. A.—Specimen 5204. Peritoneum Bovine. Natural size. Actinomycotic granulomata (Actinomycomata). Some shown on section.

Actinomycosis.]

[De Kock and Fourie.

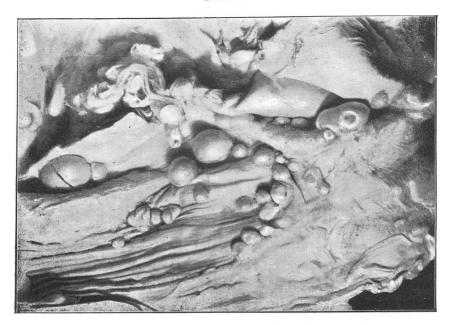
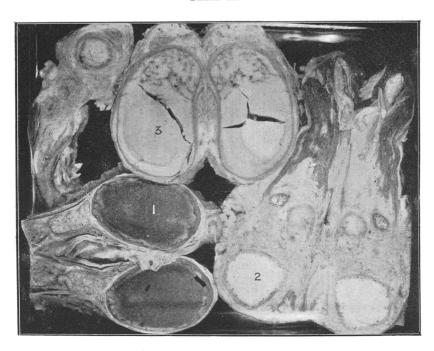


Fig. B.—Specimen 5204. Peritoneum Bovine. Natural size. Actinomycotic granulomata (Actinomycomata.)

#### PLATE II.



Specimen 5435. Actinomycosis-necrosis testicles Bovine. 1 and 2 Necrosis. 3 Necrosis and Actinomycosis. Actinocomysis.] [De Kock and Fourie.

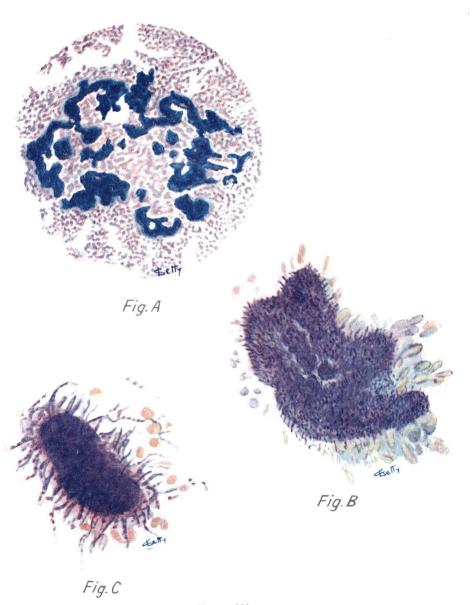


PLATE III.

Specimen 5209.—Stained Gram-weigert, shows:-

- A. Low magnification;B. Slightly higher magnification;
- C. Oil immersion.

Shows actinomyces hyphæ, branching and formacion of granules and clubs.

Actinomycosis.]

De Kock and Fourie.