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### COMMUNICATIONS

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#### TWO CASES OF GRANULOMA INVADING THE ORBIT DUE TO AN ASPERGILLUS

BY

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ON December 21, 1924, a man was sent to our out-patient department from another hospital for sarcoma of the orbit. The patient, a male Hindu, aged 35 years, presented the appearance shown in photograph No. 1. A large mass of growth bulged forward from the orbit obscuring the ordinary surface-markings except in the superior temporal quadrant. There was marked proptosis, the eyeball being displaced upwards and outwards so that the cornea lay under cover of the upper lid in the superior temporal region. The skin overlying the mass was infiltrated and adherent, dull red and glazed in parts, with several superficial breaches due to ulceration, and one or two sinuses discharging pus. Exploration of the sinuses with a probe showed that the inner wall and roof of the orbit were partially destroyed and that the growth involved these regions extensively. The nasal fossa was almost occluded, the middle turbinate being enormously enlarged. One or two yellow points showed under the mucous membrane, but there was no extensive ulceration. At first sight the condition might easily have been allowed to pass as a malignant growth with ulceration and secondary infection, but it was unusual and called for further investigation. An accurate history could not be obtained; the patient merely

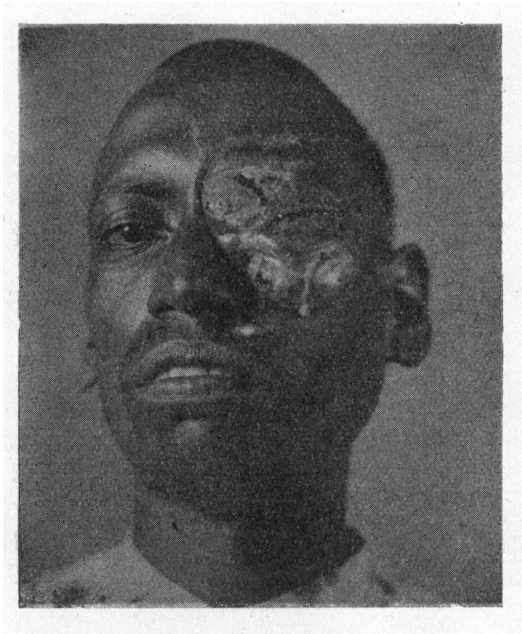


FIG. 1.

FIRST CASE.—Appearance shortly after admission.

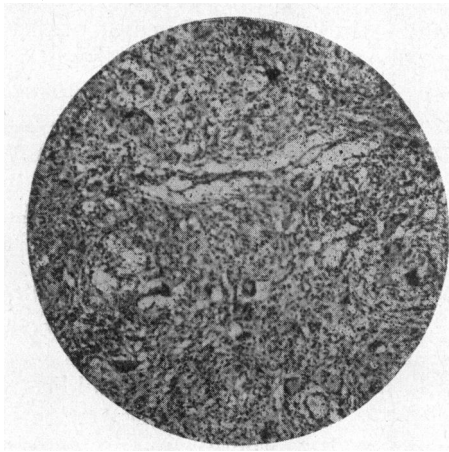


FIG. 2.

FIRST CASE.—Appearances under the low power.

asserted that it had been growing for a long time. A portion of tissue was removed for microscopic examination, and cultures taken from the pus, which was moderately thin and uniformly yellow. The pus gave a very mixed growth on ordinary media which did not prove of any importance. The sections showed a vascular granulation tissue studded irregularly with giant cells. Endothelial cells formed the bulk of the framework which was permeated by polymorphonuclear leucocytes. Fibrous elements

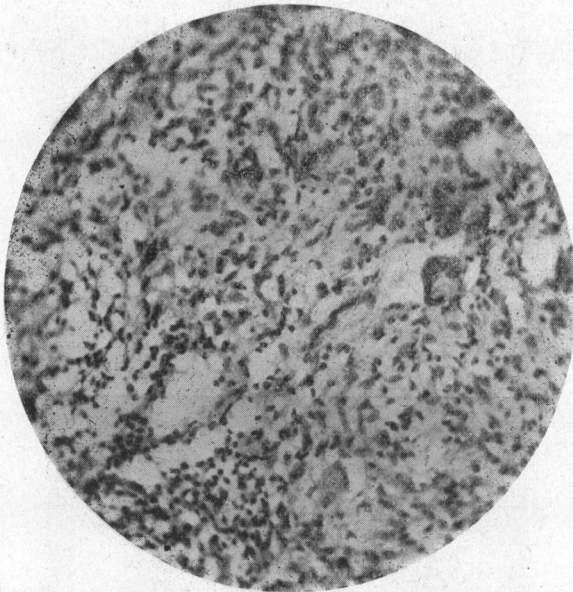


FIG. 3.

FIRST CASE.—Appearances under the high power.

were not prominent (see microphotograph appearances under the low power). Examination with the high power showed extensive endothelial activity with the production of irregular giant cells. Neither acid-fast organisms nor other parasitic agent could be seen. The appearance suggested a chronic irritant with a foreign body giant cell response, and in some respects reminded me of tissue changes which I had previously observed in investigating granulomatous conjunctivitis of the Parinaud type. This determined a prolonged histopathological investigation to discover, if possible, whether an obscure parasite was responsible. Many different methods of staining were tried

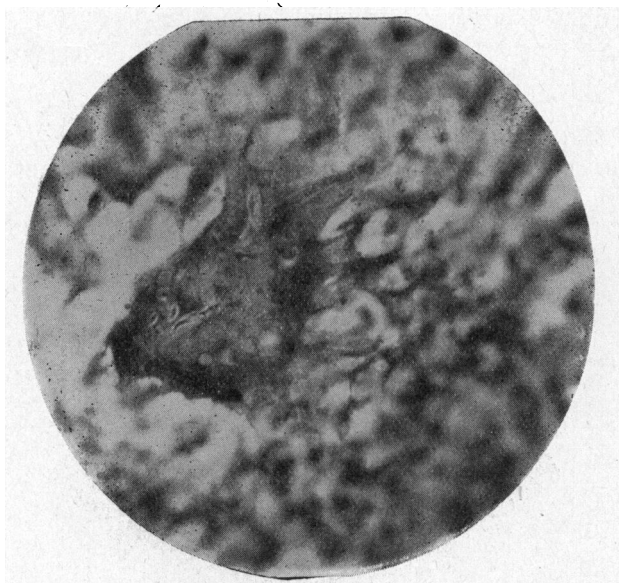


FIG. 4.

FIRST CASE.—Giant cell showing septate mycelial filaments in a giant cell. (Oil immersion lens; Leishman's stain.)

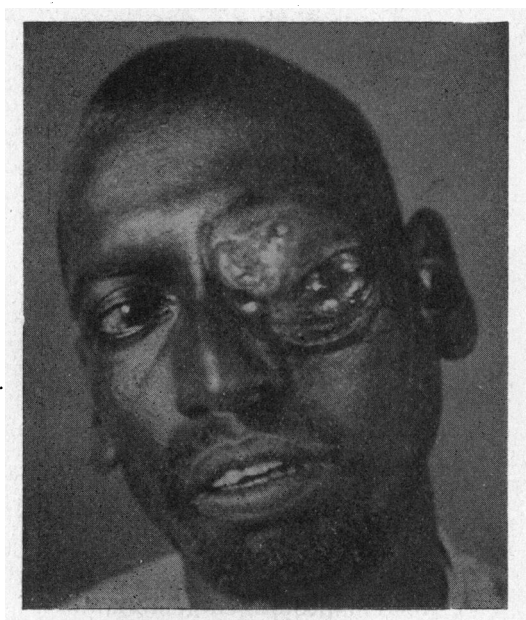


FIG. 5.

FIRST CASE.—Appearance of patient after sixth X-ray exposure.

and numerous sections examined till eventually I noted a characteristic appearance in a giant cell: the unstained outline of a filament of a coarse septate mycelium showed up clearly against the stained protoplasm. Portions of tissue had been submitted to several pathological laboratories, but no satisfactory report as to the nature of the condition was obtained, which serves to show how difficult it was to detect the fungus. On referring my observation to Lt.-Col. Cornwall, I.M.S., Director of the Pasteur Institute of Southern India, he confirmed the presence of mycelial filaments in the giant cells. Subsequently I was able to stain specimens both with Leishman's stain and haematoxylin fit for microphotographic record (see microphotograph), and to obtain pure cultures from the granuloma.

Pending further investigation the patient was put on large doses of potassium iodide and arsenic, but this treatment did not seem to alter his condition. He was then treated by X-rays and slowly and steadily improved (see photograph taken after sixth exposure). This improvement continued until it was considered that the condition might be eventually cured. At this stage the patient decided to leave hospital and could not be persuaded to stay. Some months later he returned in almost the same condition as when first seen. Again he was treated by X-rays, but went away somewhat improved after a few exposures.

#### Cultivation of the fungus

The tumour was incised through sterilized unbroken skin and a portion removed from its depth with all due bacteriological precautions against contamination. The piece of growth was divided into numerous small fragments and planted out on Sabouraud's medium, solid and fluid, maltose, glucose and glycerine. Tubes of each variety were incubated aerobically and anaerobically. Some thirty tubes were planted and in about twenty a pure growth of the fungus was obtained. The growth was more profuse in the tubes incubated aerobically. The anaerobic glycerine agar tubes showed no growth. The photographs of the growth on solid media, after 72 hours, showed the nature of the original cultures. A dense white felted wrinkled growth spread out all round the tissue and into the depth of the medium. In older cultures the surface of the growth became covered with white down-like hyphae which eventually turned yellow, then greenish and finally brown. In the broth tubes a fluffy growth developed round the tissue at the bottom of the tube while fine eiderdown-like colonies appeared in the clear fluid and on the sides of the tube.

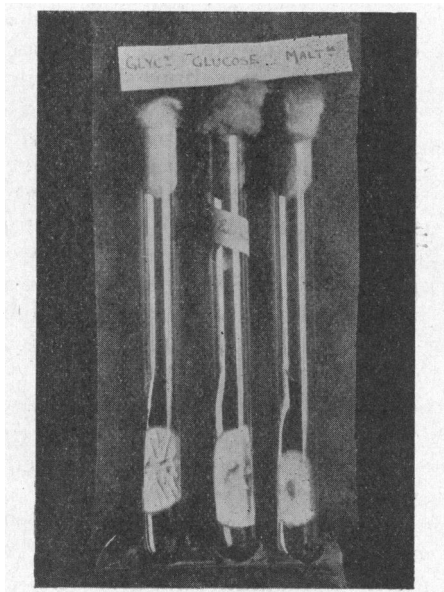


FIG. 6.

FIRST CASE.—Original cultures on Sabouraud's glycerine, glucose, and maltose agar (aerobic) after 72 hours.

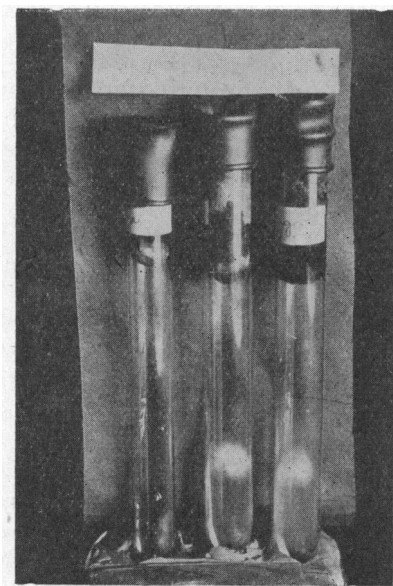


FIG. 7.

FIRST CASE.—Original cultures on Sabouraud's glycerine, glucose, and maltose agar (anaerobic) after 72 hours.

### Intravenous inoculation of pigeons with the fungus

These were carried out in the following way. A portion of a week-old growth on glucose agar, both from the surface and the depth of the medium, was emulsified and a few minims inoculated into the axillary vein of a pigeon; it remained healthy. A twenty-four hour glucose broth subculture from an original pure culture of the fungus was shaken up and used to inoculate two pigeons: one received 1 c.c., and the other 0.25 c.c., intravenously. Both remained healthy. Apparently the fungus was non-pathogenic for pigeons. (Unfortunately in the extract of this work given in my Annual Report of the Government Ophthalmic Hospital for 1925 there is a printer's error. In dealing with intravenous inoculations of pigeons it reads: "did produce aspergillosis." It ought to read: "did not produce aspergillosis.") I did not pursue the investigation of the parasite further, but sent original cultures to Lt.-Col. Cornwall, I.M.S., who kindly furnished me with the following note:

"The original cultures sent to me grew from pieces of the tumour sown directly by Major Wright on Sabouraud's maltose, glycerine and glucose agar tubes. The original cultures did not produce conidiophores. Subcultures were at once made on maltose, glycerine and glucose agar tubes having a reaction of +2 on Eyre's scale, that is, the media were slightly acid. Conidiophores formed in all the subcultures. Maltose and glucose suited the fungus better than glycerine. It grew well on potato, but the formation of conidiophores was restricted. The optimum temperature was about 37° C.

Alkalinity and greater acidity than +2 Eyre's scale checked growth. The fundus is aerobic in habit.

#### *Microscopic appearances.*

The mycelium formed a snowy white felt on the surface of the medium.

In favourable circumstances conidiophores developed in about 48 hours.

The heads were at first colourless, then became light greenish-yellow, turning later to olive-green and finally to a brownish hue.

After about 14 days incubation, black perithecia of different sizes, from 0.5 mm. to 1 mm. in diameter, appeared in some culture tubes.

On an agar slope the thinner, drier, upper third bore the bulk of the conidiophores; the middle third bore perithecia and fewer conidiophores; the lower third was covered only by white mycelium.

#### *Microscopic appearances.*

Mycelium: Smooth, colourless, branched, septate. Diameter of threads 3 $\mu$  to 5 $\mu$ .

Conidiophores: Mammillated surface, colourless, septate.

Length 4 mm. to 8 mm., diameter 6 $\mu$  to 10 $\mu$ .

Sterigmata: Mostly simple, some with a short lateral branch.

Conidia: Spherical, slightly elliptical or ovoid in outline. Surface mammillated, faintly coloured,  $3\mu$  to  $4\mu$  in diameter.

Perithecia: More or less spherical; blackish-brown, rough surface. Casing hard.

*Pathogenicity.*

A rabbit was inoculated in the posterior auricular vein with a suspension in saline of conidiophores and conidia derived from a few square millimetres of a glucose agar culture. On the fourth day its respiration was embarrassed and it died on the fifth day. Cultures were made in maltose, glucose and glycerine broth tubes from some of its organs with the following result:

Heart blood	Pure culture of the aspergillus.
Lung	„ „ „
Liver	„ „ „
Spleen	„ „ „
✓ Kidney	„ „ „

Microscopically the lungs showed signs of lobular pneumonia and the bronchi were full of secretion; the liver was congested; the spleen was apparently normal; the kidneys were spotted with whitish irregular areas both on the capsular surface and deep in the substance.

The aspergillus is pathogenic for the rabbit when its conidia are injected intravenously in a moderate dose.

The aspergillus conforms most closely to the descriptions of aspergillus flavus, but I can find nowhere sufficiently detailed descriptions of this and several other species to be quite certain that it is not an undescribed species.

I have examined many sections of the perithecia but have not yet found one in which ascospores have developed, so I am as yet unable to give a description of the shape and appearance and number of the ascospores.

I enclose a photograph of a section through a conidiophore."

In addition to the above note Lt.-Col. Cornwall furnished me with a photograph of the fructification taken from the subcultures. I subsequently made inquiries as to whether he had utilized pigeons in testing pathogenicity and he kindly gave me the following information:

"Fresh cultures were grown from original spores on October 26, 1925. The fungus grew readily, but formed perithecia in a couple of days instead of two or three weeks as happened in the originals. I gave two pigeons an intravenous injection of spores from the old tubes and two from the new tubes. They remained healthy. I did not try pigeons originally. Either they are immune or the fungus has lost its infective power in culture."



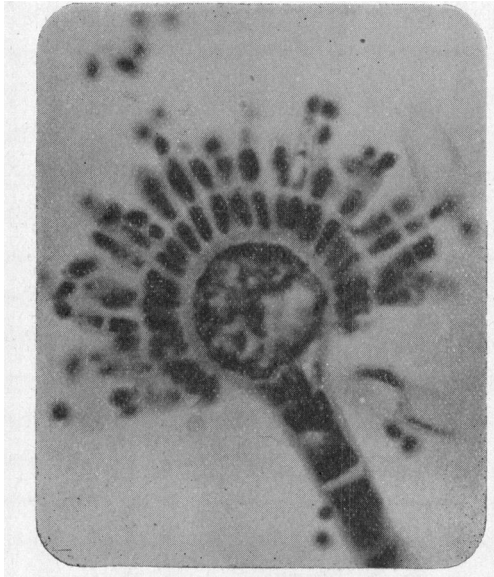


FIG. 8.

FIRST CASE.—Section through a conidiophore X 362.

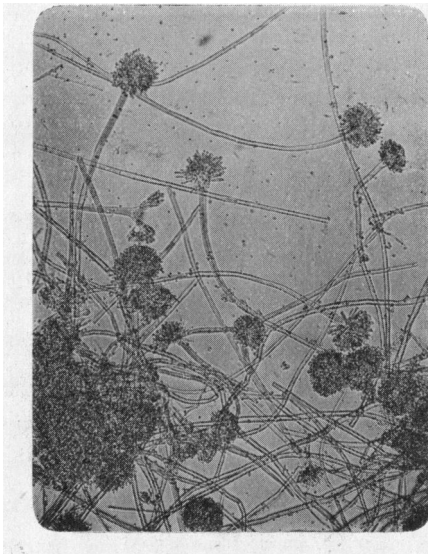


FIG. 9.

FIRST CASE.—The fructification.

The second case was that of a woman who came to the out-patient department on November 20, 1925, because of two small hard elevations about the size of large peas under the skin above and below the right internal tarsal ligament. She said they had been there for one and a half years, but the history is doubtful. The skin moved over them and there was no obvious inflammation. There was chronic dacryocystitis with complete obstruction. On examination of the nose on the same side there appeared to have been considerable destruction of the mucous membrane about the sphenoidal and posterior ethmoidal regions, where it was replaced by scar tissue. The anterior end of the middle turbinate was oedematous and there was granulatous tissue in the superior fossa. There was no suppuration, the appearances were rather those of an old granulatous process which had spread slowly and had for the most part healed leaving extensive scar tissue behind; it suggested a syphilitic condition.

Apparently the process was still in progress towards the anterior part of the superior meatus. The history was not helpful. A curved incision was made on the mesial side of these nodules with the idea of elevating them together with the periosteum and the lacrymal sac, to find out, if possible, whether they were connected in any way with the sac. The periosteum was elevated and it was found that these two nodular growths abutted on the nasal processes of the frontal bone and superior maxilla. They appeared to be incorporated with the periosteum and the underlying bone was rough. They were continuous with indurated tissue extending into the nasal cavity. On cutting into one of them it was found to be solid in nature. A small fragment was removed and when examined histopathologically was recognized to be very similar in structure to the tissues obtained from the above-mentioned case, so much so, that I did not hesitate in diagnosing a granuloma of fungoid origin (see photographs). It took me some time, however, to find the aspergillus in the giant cells as even with previous experience of staining for these parasites it was only with the greatest difficulty that a characteristic appearance was obtained. Meanwhile, however, pure growths had been obtained on a number of tubes by taking small fragments from the depth of the two nodules and planting tiny portions on tubes of Sabouraud's medium. The nodules in this case showed no inclination whatever to necrosis or suppuration, and were evidently very slow growing. The patient was sent for X-ray treatment, but did not appear to improve in the same way as the first case. What was left of the nodules remained practically the same size after the X-ray exposures. The woman, however, did not stay long enough for us to reach a final decision on this point. She took her discharge and unfortunately we have not

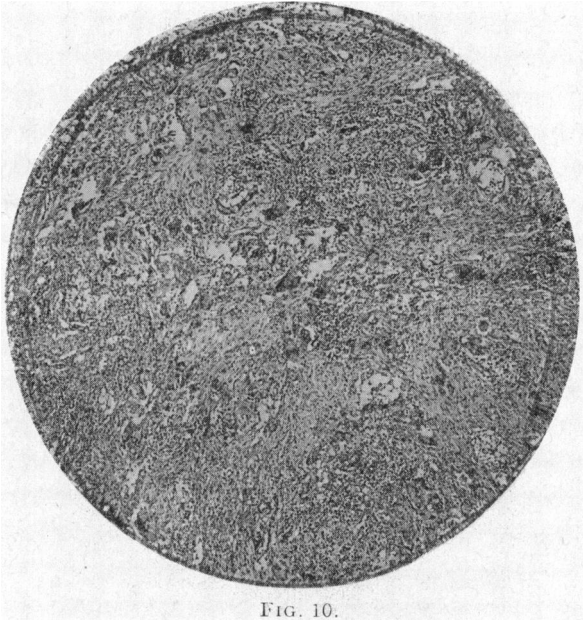


FIG. 10.

SECOND CASE.—Appearances under the low power.

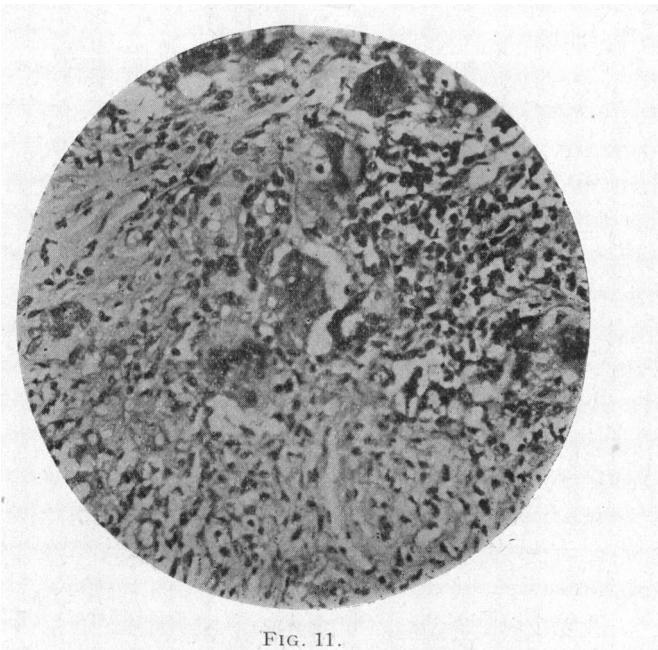


FIG. 11.

SECOND CASE.—Appearances under the high power.

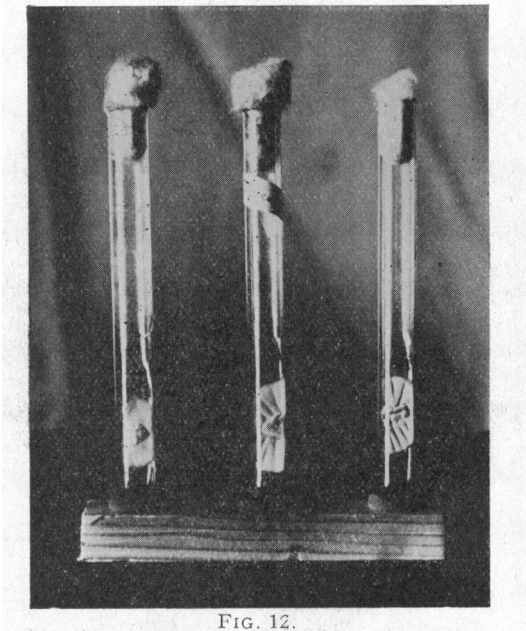


FIG. 12.

SECOND CASE.—Original cultures on Sabouraud's glycerine, glucose, and maltose agar (aerobic).

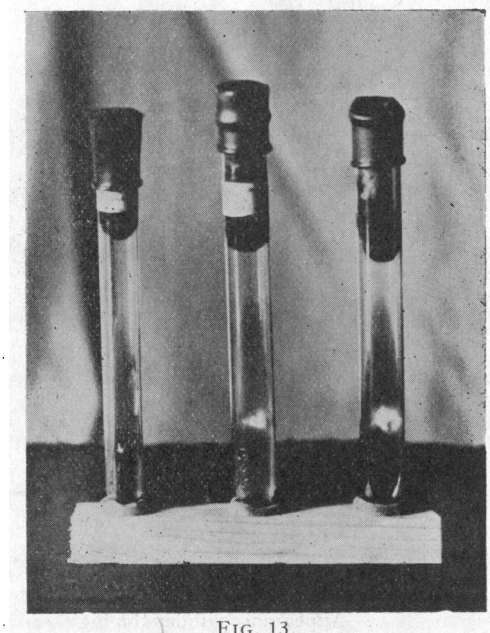


FIG. 13.

SECOND CASE.—Original cultures on Sabouraud's glycerine, glucose, and maltose agar (anaerobic).

been able to trace her. One got the impression that the nodules formed the anterior extension of a process which had started in the nasopharynx and slowly advanced leaving a trail of cicatricial tissue. The dacryocystitis was secondary to pressure of the nodules on the duct.

#### **Observations on pathogenicity, second case**

On January 16, 1926, a large number of tubes of Sabouraud's medium was inoculated with small portions of the tissue which had been removed with all due precautions to ensure sterility. These were despatched to the King Institute, Guindy, for incubation and report. Practically all the tubes showed a pure culture of fungus. They were indistinguishable from the growths seen in the first case. The appearance is shown in the photographs. From the original tubes I made subcultures on Sabouraud's glycerine agar and after incubation for 96 hours these were used for animal inoculation. A suspension of the surface growth was made in sterilized saline. The saline was run into the tube and the surface of the medium stirred up with a glass pipette. Possibly some of the deep growth in the medium may have been included in the suspension, but for the most part it contained green powdery surface growth. Two rabbits and four pigeons were inoculated. They each received one c.c. of suspension intravenously, but all remained alive and healthy. Evidently pigeons and rabbits were not affected by massive intravenous inoculations of surface growth suspension from 96-hour old subcultures. This does not, however, disprove the pathogenicity of the fungus for rabbits and pigeons. Suspensions of the original growths or even old subcultures or suspensions definitely including deep growth into the medium might have given positive results. It was impossible for me to undertake further work with the fungus here and as Lt.-Col. Cornwall had left India, I requested Lt.-Col. J. Cunningham, I.M.S., Director of the Pasteur Institute, Kasauli, to take over some of the original growth from both cases and try to work out the fungus should he have time to do so. Subsequently tubes were transferred to the Tropical School of Medicine and Hygiene, Calcutta, where Major H. W. Acton, I.M.S., is actually engaged in research in connection with pathogenic moulds and fungi and he hopes that he may be able to give this particular fungus a definite position.

#### **References to literature**

The literature on the subject of the aspergilli is very extensive and confusing. Authors vary considerably in their ideas as to what may be included under the term "Aspergillosis." As

Lt.-Col. Cornwall wrote when I asked him to work up my cultures : "the genus has not been reduced to order ; so classification of species is a matter of some difficulty." He furnished me with over 140 references from the library of the Pasteur Institute of Southern India and one can safely say that none of them dealt with granulomatous changes at all comparable with those met with in my cases. In addition to the above references I have to thank the librarian of the Royal Society of Medicine for looking up the subject and furnishing me with extracts of articles bearing upon it. As a result of his extensive search very little was found on human tumour formation due to aspergilli. It would serve no useful purpose to quote at length the number of references gone through ; these will no doubt be dealt with in the proper place when the fungus is investigated. In going through the ophthalmological literature at my disposal I found only one reference to a condition which appears to be similar to the cases described above. Dalmeyer described an inflammatory growth involving the orbit and base of the brain, discovered post-mortem. (*Zeits. f. Augenheilk.*, Bd. 31, S. 420.) I have not consulted the article in the original. The bulk of the reports on aspergillus deal with the condition as it forms a surface growth on skin or cornea, in the external auditory meatus, the nasal sinuses, air passages and lungs (pseudo-tuberculosis), or as small deposits in the internal organs. Obici in a long series of extracts from various authors quotes Rupert Boyce's case in which the apex of the right lung was involved.

The bronchi were invaded and there were nodules in the lung which showed "numerous macrophages which submerged and enclosed the hyphae." (Obici, Augusto: *Ueber die pathogenen Eigenschaften des Aspergillus fumigatus—Beitr. z. path. Anat. u. z. allg. Path.*, Jena, 1898, Bd. XXII, SS. 197-237.) This is the only mention I can find suggestive of the cellular response to the fungus as seen in my cases. In similar infections, however, this has been noted. In blastomycotic dermatitis (Gilchrist) the fungus responsible has been described in the giant cells. (Dowling and Elworthy: *Proceedings of the Royal Society of Medicine*, Vol. XIX, No. 2, December, 1925; Section of Dermatology, page 8.) Apart from membranous and ulcerative lesions of the throat and nose, bronchi and lungs, eye and ear, and nodular deposits in the internal organs, large granulomata resembling Madura foot have been described. For example Nicolle and Pinoy describe a condition clinically very similar to the ordinary mycetoma met with in South India due to actinomyces (discomyces). Nicolle and Pinoy: *Sur un cas de mycétome d'origine aspergillaire observé en Tunisie. Arch. de Parasitol.* T.X, pp. 437-440, 457-8, 1905.) They isolated a fungus different from

*discomyces madurae* (Vincent) which was investigated by the Pasteur Institute, Tunis, and pronounced to be *aspergillus* (*sterigmatocystis*) *nidulans*. A description of the histopathology is not given. They considered that possibly *aspergillus* (*sterigmatocystis*) *nidulans*, or some closely allied fungus may be the pathogenic agent in mycetomata not due to *discomyces*. This may be so, but there are other workers in this field who consider that *aspergillus* may be a secondary implantation on a primary *discomycotic* condition. In very many articles dealing with *aspergillosis*, *A. fumigatus* is incriminated; sometimes apparently without very much reason, as diagnostic characters are not given. Even if they were, it would perhaps be difficult to place such fungi as the characters of the group have been insufficiently worked out. It is certain, however, from the literature that fungi of the genus *aspergillus* are responsible for both saphrophytic and parasitic invasions of man and animals and that they may cause not only surface infections but definite granulomata. There can be no doubt from the histopathology of the granulomata in my cases that the *aspergillus* was a true parasite and almost certainly acting primarily. In the second case there was no evidence whatever that there were organisms of any other sort present in the tissue. In the first case there were bacilli and cocci, but they were in all probability those of a secondary infection.

My thanks are due to Mr. H. Hawley of the King Institute of Preventive Medicine, Guindy, for the microphotographs, and to the South Indian Branch of the British Medical Association for meeting the expenditure associated with the animal experiments.

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### THREE CASES OF EMBOLISM OF A RETINAL ARTERY

BY

T. HARRISON BUTLER

BIRMINGHAM

C.J.W., male, aged 21 years, came to the Birmingham Eye Hospital on December 7, 1926, and was examined at 10 a.m. He stated that at 7.40 a.m. the vision of the left eye suddenly failed and that for some time he had not the slightest perception of light. In about half an hour he began to see again, but even now the sight is not normal. He suffers from morbus cordis.

The vision of each eye is 6/6. There is a quadrantic notch in the field of vision involving the lower temporal aspect, as shown in the chart.